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Development of An Optimal Sol-Gel Immobilization Process for Uranium Adsorbing Biomass

A Final Report to:

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TABLE OF CONTENTS

Page No.

EXECUTIVE SUMMARY 1
1. INTRODUCTION 2
2. EXPERIMENTAL 3
Phase I - Preparation of Sol-Gel Biomass 3
Phase II - Column Preparation 3
Phase III - Loading and Elution Optimization 3
2.1 Preparation of Sol-Gel Biomass 4
2.1.1 Biomass Production 4
2.1.2 Cell Wall Isolation 6
2.1.3 Sol-Gel Biomass Production 6
2.2 Column Preparation 8
2.2.1 Sol-Gel Biomass Comminution 8
2.2.2 Stir Tank Tests 9
2.2.3 Column Set-up and Operation 10
2.3 Loading/Elution Optimization 11
2.3.1 Breakthrough Points 11
2.3.2 Flow Rate Optimization 11
2.3.3 Size Distribution 11
3. RESULTS 12
3.1 Preparation of Biomass 12
3.2 Sol-Gel Biomass Production 13
3.3 Column Preparation 15
3.4 Loading and Elution Optimization 18
4. DISCUSSION 20
5. CONCLUSION 24

TABLE OF CONTENTS CONT'D.

Page No.

TABLES 26

Table 1 Protein Concentrations Resulting From Osmotic Shock of Rhizopus arrhizus 27

Table 2 Sol-Gel Biomass Compositions and Comminutions 28

Table 3 Preconditioning 29

Table 4 Size Distribution 30

Table 5 Elution Characteristics 31

FIGURES 32

Figure 1 Growth of Rhizopus arrhizus in the Braun Biostat V Fermentor as Monitores by pH 33

Figure 2 Protocol for Osmotic Shock of Rhizopus arrhizus Cells 34

Figure 3 Effect of 3 Stir Tank Loading Elution Cycles on (+28/-20) Sol-Gel Particles (with 1 g fines, 5 g biomass) Elution pH 35

Figure 4 Effect of 3 Stir Tank Loading Elution Cycles on (+28/-20) Sol-Gel Particles (with 3 g fines, 5 g biomass) Elution pH 36

Figure 5 Effect of 3 Stir Tank Elution Cycles on (+28/-20) Sol-Gel Particles (with 5 g fines, 5 g biomass) Elution pH 37

Figure 6 Effect of 5 Load/Elution Stir Tank Cycles on U-uptake of (+20/-14) Particles (with 1 g fines, 5 g biomass) and Loading Effluend pH 37

Figure 7 Effect of 5 Load/Elution Stir Tank Cycles on U-uptake of (+20/-14) Particles (with 3 g fines, 5 g biomass) and Loading Effluent pH 39

TABLE OF CONTENTS CONT'D.

Page No.

Figure 8 Effect of 5 Load/Elution Stir Tank Cycles on U-uptake of (+20/-14) Particles (with 5 g fines, 5 g biomass) and Loading Effluent pH....40

Figure 9 Effect of Load/Elution Stir Tank Cycles on U-uptake of Pure Biomass and Loading Effluent pH....41

Figure 10 Effect of 8 Stir Tank Cycles on Uranium Uptake of (+20/-14) Sol-Gel Biomass Particles and Solution pH....42

Figure 11 Effect of 10 Loading Elution Stir Tank Cycles on Uranium Uptake of (+28/-20) Sol-Gel Biomass Particles and Solution pH....43

Figure 12 Effect of 10 Load/Elution Stir Tank Cycles on U-uptake by +28/-20 Preconditioned Sol-Gel Biomass (with Ethanol Formation) and the Effluent pH....44

Figure 13 Column Loading Effluent pH Profile of (+28/-20) Sized Biomass Sol-Gel For One Cycle (2550 mL) (Eluting with 5% Na_2CO_3 Caused Plugging)....45

Figure 14 Column Loading Profile (+20/-14) Sol-Gel Particles For One Cycles Before Plugging Occurs....46

Figure 15 Column Loading Profile (+20/-14) Sol-Gel Particles For One Cycles Before Plugging Occurs....47

Figure 16 Effect of 8 Load/Elution Cycles in a Column on U-uptake by +28/-20 Sol-Gel Particles Without Ethanol or Fines in the Formation....48

Figure 17 Effect of 8 Load/Elution Cycles in a Column on U-uptake by +20/-14 Sol-Gel Particles with 5 g fines/5 g biomass but no Ethanol in the Formation....49

Figure 18 Effect of 8 Load/Elution Cycles in a Column on U-uptake by +28/-14 Sol-Gel Particles with 5 g fines/5 g biomass and 5 mL Ethanol/10 g solids in the Formation....50

REFERENCES....51

CONTRACT PLAN AND REPORT FORM....52

EXECUTIVE SUMMARY

The objective of this study was to test and optimize a new sol-gel formulation. This is to be used in conjunction with the unproven industrial waste-grown Uranium adsorbing fungus Rhizopus arrhizus. The testwork was undertaken on a bench scale in an attempt to simulate an ion exchange process on an industrial scale. Sol-gel formulation was optimized with respect to fungal form used, the addition of fines, and the effect of preconditioning. Uranium loading and elution optimization included particle sizing, and the effect of flow rates. The U-uptake values were highest at 60 mg/g biomass and observed in stir tests using sol-gel composed of 50% comminution fines. When ethanol was reduced or removed from the composition of the sol-gel, particle size degradation was lowered, while U-uptake values fluctuated between 10 and 40 mg/g biomass dependant on particle size and formulation. The column tested sol-gel indicated a lifetime of at least 8 loading elution cycles which has the potential for an extended lifetime upon further optimization.

RÉSUMÉ EXÉCUTIF

L'objectif de cette étude fut d'examiner et d'optimiser une nouvelle formule chimique de sol-gel. Cette nouvelle formule doit être utiliseé en conjonction avec l'organisme fongique Rhizopus arrhizus. Cet organisme se prolifére dans les déchets industriels et adsorbe l'Uranium. Il n'est toutefois pas prouve' qu'il puisse adsorber l'Uranium dans cette nouvelle formule de sol-gel. Le travail experimental fut entrepris à une e'echelle d'étude de laboratoire pour tenter de simuler un processus d'échange d'ions sur une échelle industrielle. La formule de sol-gel fut optimisée en ce qui concerne l'espèce fongique utilisée, l'adjonction de fines particules poudreuses, et l'effet de préconditionnement. L'optimisation du chargement et de l'extraction de l'Uranium inclut la se'paration de particules par tailles et les effets de la vitesse d'écoulement. Le montant d'Uranium extrait par le sol-gel fut le plus élevé à 60 mg/g de biomass et fut observé dans un test où la substance fut remueé, utilizant un sol-gel composé de 50% de fines particules séparées. Quand le montant d'éthanol fut réduit ou exlus de la composition de sol-gel, la dégradation de la taille de particules fut diminuée a lors que le montant d'Uranium extrait fluctua entre 10 et 40 mg/g de biomass, dépendant de la taille des particules et de la formule chimique. Le sol-gel testé à l'aide d'une pipette démontra avoir longérité d'au moins 8 cycles de chargement-extraction ayant donc le potential d'une longévité prolongée si le sol-gel est optimisé davantage.

1. INTRODUCTION

Recent developments in utilizing fungal biomass to recover uranium from underground bioleach operations have demonstrated the potential integration of high performance and economy. A high performance immobilization procedure (developed under DSS contract 14S2.23440-7-922), and the development of a fungal growth medium using an economical carbon based food industry waste (under DSS contract 23440-8-9198/01-SQ) have give potential for the use of non-specialized equipment in U-extraction procedures. The objective of this study is to optimize the sol-gel formulation as a biomass immobilizing method for Rhizopus arrhizus in order that maximum stability be obtained with loading, elution and regeneration cycles.

2. EXPERIMENTAL

The experimental work is divided into three phases, with tasks assigned to each phase as described:

Phase I - Preparation of Sol-Gel Biomass

Task 1.1	Biomass Production
Task 1.2	Cell Disruption, Cell Wall Isolation
Task 1.3	Sol-Gel Biomass Production

Phase II - Column Preparation

Task 2.1	Sol-Gel Biomass Comminution
Task 2.2	Stir Tank Tests
Task 2.3	Column Set-up

Phase III - Loading and Elution Optimization

Task 3.1	Loading/Elution Breakthrough Points
Task 3.2	Flow Rate Optimization
Task 3.3	Size Distribution

2.1 Preparation of Sol-Gel Biomass

2.1.1 Biomass Production

Based on the results of a previous investigation (DSS 23440-8-9198/01-SQ), the uranium adsorbing fungus Rhizopus arrhizus was produced using the industrial food waste, spent brewing grain, from Connor's Brewery (Toronto) as a source of nutrient growth medium. To obtain a suitable medium for growth, the spent brew grain was subjected to a water extraction process. Four hundred grams per litre tap water (400 g/L) were boiled for one hour, filtered through three (3) layers of cheesecloth to remove the coarse brew grain, then filtered again using vacuum Buchner filtration through a #541 Whatman filter to remove the finer particulate matter.

This preparation was then autoclaved in 2.25 litre quantities, in a 6 litre Erlenmeyer flask, at 121 °C, 16 psi, for 25 minutes. Streaking Tripticase-soy agar plates with the autoclaved medium resulted in no growth, indicating sterility of the medium. A precipitate, evident after autoclaving, was allowed to settle in the flasks with the supernatant being carefully decanted into the growth vessel. The growth vessel used was a Braun 6 litre Biostat V fermentor (stainless steel and borosilicate glass) fully autoclavable and capable of holding a working volume of 4.5 litres. Stirring the contents of the fermentor was achieved with the equipped centrally located 3 impellar (6 blades each) device. Oxygen was oil-filtered, humidified air.

Growth of the fungus was initiated with preparation of the spore inoculum. Sterilized Potato Dextrose Agar was dispensed in 15-20 mL portions then inoculated with 0.1 mL of a light spore suspension (prepared by agitating sporangiophores into a volume of 0.1 M phosphate buffer pH 5.8, then passing through sterilized cotton batten). Six petri dishes of cultured, sporulating Rhizopus arrhizus would supply enough inoculum for 2 fermentations. A final inoculum concentration of 2.5×10^3 spores per mL growth medium was kept constant using the cell counting method of the Neubauer Haemocytometer. Maintaining aseptic transfer techniques, 4.0-4.25 L growth medium, inoculum, as well as 3 mL corn oil (separately autoclaved) as anti-foam agent were put into the fermentor. The stir speed and aeration rate were progressively increased to maximum during the initial 12-15 hours of the growth period, culminating in the harvest of the biomass 24-30 hours after inoculation. The physical conditions of temperature and pH were monitored during growth, with a pH of 3.0 ± 0.10 pH unit indicating the end of the log phase of growth and the point of harvest. Morphological observations at 400X power using light microscopy were conducted during pH sampling to follow the growth habit of the fungus as well as monitor for bacterial contamination.

When harvest was necessary, the biomass was filtered through three (3) layers of cheese cloth, washed twice in tap water (4 L each), gently squeezed to remove water, then frozen until four such quantities of biomass were obtained for freeze drying.

2.1.2 Cell Wall Isolation

Attempts were made to increase the efficiency of the uranium adsorptive process by isolating the cell walls, the site of U-adsoption (Tsezos, Volesky, 1982). On advice from the Scientific Authority, osmotic shock of the organisms using sucrose at various concentrations was initiated. Sucrose concentrations of 0.73M (25%) and 1M were used as well as acetone (C.Booth, 1971) to try and break the cell walls, enabling the cytoplasm of leak out. After suspending a measured quantity of cells in the osmotic medium for 15 minutes, the cells were centrifuged, the aqueous sampled, the cells suspended in 50 and 150 mL volumes of distilled water for 15 minutes and then filtered through a 0.45 µ Millipore filter, with the filtrate and the above aqueous being sampled for protein estimation by ultraviolet absorption (Layne, 1957). Acetone at reagent strength was used in the same manner, however, the Millipore filter was dissolved and it was impossible to obtain a UV absorption at 260 mm or 280 mm. Visual examination at 400x light microscopy for evidence of disrupted cells was conducted on the samples following the distilled water wash or the acetone suspension.

2.1.3 Sol-Gel Biomass Production

Based on results obtained in a previous DSS sponsored investigation (DSS 145Q 23440-6-9042), a sol-gel formulation to immobilize fungal biomass was used. The formation of the basic sol-gel biomass compound was as follows:

Stir (5 minutes) 10 g freeze dried biomass and 100 mL Na_2SiO_3 (2.5% w/v), then add 50 mL $AlCl_3$ (5% w/v) and 15 mL reagent grade ethanol (denatured) and stir an additional one minute. Vacuum filter using the Buchner and #541 Whatman paper and dry at 85°C for 2 hours or until brittle. Variations on the basic sol-gel formulation were incorporated using finely ground fines obtained from the comminution of the sol-gel fines biomass. Biomass and fines combinations of 5 g biomass plus 5 g fines using the above proportions,.and 5 g biomass plus 3 g fines and 1 g fines in half-formula aqueous proportions constituted the variable of fines incorporation. Freeze dried biomass was pulverized to a powdery consistency prior to sol-gel formulation, but sizing was not possible due to the poor screening characteristics of pure dry biomass. A comparison of oven dried biomass to freeze dried was made. The biomass was oven dried at 65°C for 48 hours and subsequently powderized and incorporated into sol-gel as above.

Preconditioning the sol-gel biomass with alternate carbonate-acid treatment was used to try and limit degradation of the sol-gel and plugging of the column. Seven (7) grams of sol-gel biomass was stirred for one (1) hour in 150 mL Na_2CO_3 (10% w/v), filtered (#541 Whatman) and washed with 100 mL distilled water, washed twice with 50 mL pH H_2SO_4, washed again with 10 mL Na_2CO_3 (10% w/v), washed twice with 100 mL distilled water, then washed twice with 30 mL denatured ethanol, before being dried for one hour at 85°C.

An alternate preconditioning method was employed to try and limit plugging of the columns during loading-elution of the sol-gel biomass. Ethanol was either used in a decreased amount or not at all during the formation of the sol-gel. This method was used with a fines/biomass combination of 5g each, both ethanol adjustments being used, as well as pure biomass with ethanol absent. Instead of circulated air drying at 85°C, vacuum air drying at 85°C was used.

2.2 Column Preparation

2.2.1 Sol-Gel Biomass Comminution

To size and screen the sol-gel biomass filter cake, two (2) different methods of comminution were employed. A shatter box, consisting of two (2) concentric metal rings, one hollow, one solid, which fit into a larger round metal "box" with space between the rings for pulverizing the material, was used initially. Hand grinding, using a mortar and pestle, was ultimately selected as the method of choice for comminuting the sol-gel particles.

The comminuted particles were sized using 14 mesh, 20 mesh and 28 U.S. mesh size screens (openings of 1.41 mm, 0.84 mm and 0.60 mm respectively) to produce sol-gel particle sizes of +20/-14, +28/-20, and -28. The -28 sized fines were further pulverized in the shatter box to a finely ground powder for use in the production of sol-gel biomass incorporating fines.

2.2.2 Stir Tank Tests

Both pure freeze dried biomass and sol-gel biomass were used in stir tank tests. All the tests were agitated on a gyrotary shaker at 200 rpm and filtered where noted using a 45 micron Millipore filter. Pure freeze dried biomass was used in 0.2 g quantities contacted with 200 mL pH 4 mine water (courtesy of Ellis Green, Denison Mines, Elliot Lake, Ontario) for 10 minutes, filtered, sampled for U-analysis, then contacted with 50 mL Na_2CO_3 (5% w/v) for 10 minutes, filtered and sampled. Three five cycle runs in this manner were tested.

Sol-gel biomass (and pre-conditioned) was used in 0.2 g and 0.5 g quantities, contacted with 200 mL and 300 mL volumes (respectively) of pH 4 mine water for 10 minutes, filtered and sampled for uranium analysis, then contacted with 30 mL and 50 mL volumes (respectively) for Na_2CO_3 (5% w/v) for 10 minutes, filtered and sampled. One, three, five, eight and ten cycle runs were tested. The eight and ten cycle runs were interrupted with 10 mL distilled water washed between the minewater and carbonate contacts.

As received Denison minewater had a pH of 2.45 and required increasing to pH 4.0, accomplished with dropwise addition of a saturated NaOH solution. Following pH adjustment, the heavily precipitated mine water was allowed to settle and further decanted and filtered through a 0.45 micron Millipore unit.

2.2.3 Column Set-up and Operation

The set-up and operation of testwork involving columns was based on existing ion exchange processes, albeit on a smaller and simpler scale. Initially a 10 cm^3 tap-down volume in a 50 mL buret (1 cm width) was used with 6.23 g of (+28/-20) particles. The sol-gel particles were loaded into the pH 4 H_2O (with H_2SO_4)-filled column then washed with 10 bed volumes of distilled water (to remove extraneous protein material). Mine water at pH 4 was then pumped using peristaltic action into the column in an upflow at 0.2 L/hr with sampling for U done every 15 mL for 300 mL then every 50 mL for 2250 mL for a total of 2550 mL. Attempted elution with an upflow of Na_2CO_3 (5% w/v) followed but plugging problems developed. Further testwork was initiated to solve this dilemma.

Using Pasteur pipettes as columns, 0.5 g sol-gel material (tap down volume 1.0 mL = bed volume) was tested in a variety of ways. Increasing the loading flow rate to 0.6 L/hr to cause fluidization of the particles; 10 mL distilled water washed between loading and elution and loading; altering flow directions in conjunction with the above; and using preconditioned sol-gel biomass (as described) were all tested. Glass wool was used in the ends of the column to prevent sol-gel losses.

2.3 Loading/Elution Optimization

2.3.1 Breakthrough Points

Loading and elution profiles were examined by pumping mine water or carbonate, respectively, for a known volume at a predetermined flow rate, and sampling incrementally. Mine water, at pH 4, was pumped in both upflow and downflow directions in different tests, then sampled every 15 mL, 40 mL or 50 mL for declining U concentration in the column effluent as well as pH. Uranium elution was monitored by pumping a predetermined volume of 5% (w/v) Na_2CO_3, then sampling every 10 mL for increasing U concentration in the column effluent, as well as pH. Three (3) cycles #1, #3, #5, or 5 cycles #1 to #5 were monitored in this manner.

2.3.2 Flow Rate Optimization

The flow rate of the loading mine water was varied between 0.4 L/hr and 0.6 L/hr through the column while the flow rate of the eluting Na_2CO_3 was kept constant at 3.33 mL/min. Fluidization of sol-gel particles was noted if present or, alternatively, plugging of the column.

2.3.3 Size Distribution

Destruction and degradation of the size sol-gel particles was monitored by recovering the particles after the testwork, drying at 85°C, and screened to (+20/-14), (+28/-20) or (-28) U.S. mesh sizes. Sol-gel losses were observed by comparing the recovered fraction screened sizes to the total final recovered sol-gel with respect to the mass of the original sol-gel particles.

3. RESULTS

3.1 Preparation of Biomass

Typical growth of Rhizopus arrhizus in the Braun fermentor, as monitored by pH, is shown in Figure 1. An initial log period of about five (5) hours occurs using the minimum aeration rate and a high stir speed. The pH starts at about 5.5 (± 0.2) and decreases to about 3.0 (± 0.05), the point of harvest, as well as the initiation of the death phase. Temperature monitored through the growth ranged from room temperature (25°C) to 29°C during the log phase, with some variation (± 1°C).

Within 5-7 hours of inoculation of the spent brew grain extract with the spore suspension, the spores had begun to germinate while extensive mycelial growth had occured within 15 hours. Examination of various suspensions of growth medium revealed no bacterial contamination, as evidenced by 400x light microscopy.

Attempting to rupture the cell walls of the fungus using sucrose as the osmotic pressure medium had less than favorable results. When cells were suspended and mixed well in both sucrose then distilled water and just distilled water as control, there appeared to be no visible difference when viewed under 400x power optics. Cytoplasmic contents of the mycelial fragments contained a variety of contents in both treatments with no cell wall "ghosts" appearing. Conversely, using acetone as the cell breaking method resulted in some cytoplasmic loss leading to "ghosts" and empty looking cells.

A biochemical analysis for protein release into solution with cells subjected to osmotic shock is tabulated in Table 1. Treating 2.5 g cells (wet wt.) with 1M or 0.73M sucrose, then suspending the cells in distilled water to induce osmotic shock, produces protein concentrations of between 17 mg/mL to 80 mg/mL. Analysis of the acetone filtrates for protein absorbance at 280 and 260 nm was not possible due to the threshold of between 185-400 nm at which acetone absorbs.

3.2 Sol-Gel Biomass Production

A visual comparison of sol-gel made with freeze dried biomass versus oven dried biomass revealed noticeable differences. The freeze dried material had significantly more mass per gram biomass incorporated. An average of 82.5% of the sol-gel was biomass with the freeze dried material, while about 95% of the sol-gel was biomass with the oven dried material. A very grainy texture to the oven dried material was evident following drying while the freeze dried material was of considerably greater homogeneity.

Incorporating sizing and screening fines into sol-gel production resulted in material with a greater amount of biomass if 20% or 60% of the biomass was fines, and approximately the same (% biomass) if an equal amount of fines to biomass are incorporated. When 5 g biomass and 1 g fines were mixed, 87% of the sol-gel was biomass. When 5 g biomass and 3 g fines were mixed, 90% of the sol-gel was biomass; when 5 g biomass and 5 g fines were mixed an average of 80% of the sol-gel was biomass (Table 2).

Pre-conditioning the sol-gel biomass with alternate carbonate - weak acid treatments had physical success dependant on the method of sol-gel formation (Table 3). When 15 mL ethanol per 10 g biomass was used in the formation, pretreatment produced slimes, fines and some loss of the size particle being preconditioned. The slimes and fines were produced in the first Na_2CO_3 and H_2SO_4 treatments. These had to be removed by wet screening which isolated some of the remaining (+28/-20) or (+20/-14) particle sizes. Subsequent carbonate, ethanol and water washes did not produce this problem. However, destruction of a (+20/-14) size was as much as 94% while destruction of the +28/-20 size was 100%.

Pre-conditioning sol-gel biomass which was formed without ethanol (or with reduced amounts) had more success. When the ethanol was reduced to 5 mL (from 15 mL) the larger particle size, +20/-14, had 66% recovery but the smaller size, +28/-20, had only 5% of their respective sizes. Without ethanol in the formation, the +20/-14 size had 68% (no fines) and 50% (with fines) size recovery while the +28/-20 fraction had 56% (no fines) and 35% (with fines) size recovery. Loss of sol-gel biomass in the form of fines and slimes during the alternate preconditioning process was inevitable but considerably reduced.

3.3 Column Preparation

Comminution of the prepared sol-gel biomass into (+28/-20) and (+20/-14) particles always yielded about twice or more (+20/-14) sized particles (Table 2). Comminuting the freshly dried sol-gel slabs worked equally well whether by shatterbox or mortar and pestle method, although for relatively small amounts, as in this testwork, more control is exercised with the mortar and pestle. An average of about 40% was ground to (+20/-14) size while an average of about 20% was crushed to the (+28/-20) size.

Oven dried biomass incorporated into sol-gel had a U-uptake of 56 mg/g biomass while the freeze dried biomass incorporated sol-gel had 90 mg U/g biomass. This resulted in further testwork being done with the freeze dried material.

Three and five cycle stir tank loading/elution testing was carried out on the (+20/-14) and (+28/-20) size particles, respectively (Figures 3-8). In the 3-cycle tests, the best U-uptake (mg/g biomass) was with the sol-gel incorporating 5 g fines/5 g biomass (Figure 5) which showed increasing U-uptake greater than 55 mg/g after cycle 1. Sol-gel with 3 g fines and 5 g biomass (Figure 4) had slightly less than 50 mg/g after the first cycle and did not decrease significantly, while the 1 g fines/5 g biomass (Figure 3) sol-gel increased to 60 mg/g after relatively no uptake in the 1st cycle but showed a decreasing trend. Monitoring the pH of the loading effluents revealed similar increasing and decreasing trends as in U-uptake.

Five cycle testing of the (+20/-14) particles (Figures 6 to 8) showed an increasing trend of more than 55 mg/g in the 5 g/5 g sol-gel, a stabilizing trend of greater than 40 mg/g for the 3g/5g sol-gel, and an erratic decreasing trend as high as 68 mg/g and as low as 32 mg/g for the 1 g fines/5 g biomass sol-gel. All three tests had U-uptakes of less than 10 mg/g biomass in the first cycle then increasing as described. The first cycle pH was very near the pH of the mine water (4-4.2) but with cycle 2 increased to greater than 9, except for 5 g fines/5 g biomass which increased to 5.76.

Pure freeze dried biomass was tested in a 5 cycle load/elution stir tank treatment (Figure 9). Uranium uptake was initially lower in cycle 1 at 39.5 mg/g but maintained (except cycle 3) values above 80 mg/g and as high as 103 mg U/g biomass, while pH measurements increased and stabilized at pH 9 at cycle 2.

Eight and ten stir tank load/elution cycles with (+20/-14) and (+28/-20) particles indicate relatively erratic U-uptake profiles (Figures 10, 11). In cycles 2-8, U-uptake values increase and decrease with each successive cycle between 13 mg U/g and 24 mg U/g biomass. Cycle 9 and 10 of (+28/-20) size stabilize at about 21 mg U/g biomass. The loading effluent pH varies in each test, showing 4.2 at cycle 1 to 5-5.2 at the final cycle, on an increasing trend to cycle 7 for (+20/-14) and to cycle 7 and 8 for (+28/-20) size. In a 10 cycle load elution stir tank test of ethanol formed sol-gel (-28/-20) (Figure 12), U-uptake values never rise higher than 1.75 mg/g biomass.

Elution of the uranium loaded sol-gel in 5 cycles stir tank tests generally showed complete mass balance recovery of U-loaded to U-eluted (Table 5). The testwork with sol-gel formed from 50% fines plus ethanol was the exception (+20/-14), with U-elution values of between 4.2% to 50% of that loaded. The above tests used 30 mL Carbonate (5%) to eluted 0.2 g of loaded sol-gel particles. Three cycle testwork (not tabulated), showing similarly poor results as the above mentioned test used +28/-20 particles with various amounts of fines incorporated.

The initial tests involving column U loading and elution were plagued by plugging after the first cycle. The first test involved a 10 mL bed volume of 6.23 g (+28/-20) sol-gel particles in a 50 mL buret, at a downflow loading rate of 3.33 mL/min. (Figure 13). Uranium uptake values were 20-25 mg/g biomass with the first 15 bed volumes and continued to decrease to between 0 to 5 mg U/g biomass with 50 to 65 bed volumes. Loading effluent pH increased from a base of 4.0 to 4.20 for 40 bed volumes then decreased to a stable 4.15 ± 0.02. After contacting the column with an upward flow of 5% (w/v) Na_2CO_3, plugging was evident. A small sample (10 mL) of carbonate which had passed through the column was recovered, along with a whitish precipitate showing a pH of 6.8. Splitting the sample for pH adjustment tests, one (5 mL) sample was altered to pH 2 with 10% HCl, then altered further to pH 0.5.

The original sample precipitate did solubilize at pH 2 and after an assay for aluminum and silica showed the [Al] = 32 ppm, [Si] = 18 ppm (control [Al] = <0.3 ppm, control [Si] = 17 ppm). The pH 0.5 sample upon analysis showed [Al] = 42 ppm, [Si] = 25 ppm. Stirring 2.5 g of sol-gel biomass particles with 25 mL Na_2CO_3 (5%) for 5 minutes yielded a fine white precipitate while the aqueous portion upon assay showed [Al] = 53 ppm, [Si] = 1.6 ppm.

An increased flowrate of 10 mL/min. in a smaller column (Pasteur pipette) and through 0.5 g untreated sol-gel (+20/-14) in a 1 mL bed volume, produced results with low erratic U-uptake values, as well as plugging (Figures 14-15). Fluidization of the particles was evident during upflow of the loading mine water but upon contact with a distilled water wash of 40 mL, then elution with 50 mL 5% Na_2CO_3, a further loading was not possible due to plugging.

3.4 Loading and Elution Optimization

Determination of a breakthrough point during loading of Uranium onto the sol-gel biomass is shown in Figures 16-18. Uranium loading values (mg/g biomass), as determined by the values assayed in the column effluent, show a decrease in the 50-100 bed volume range, however, variations are present.

The decrease is generally sharp to 100 bed volumes and shows relatively low U-uptake values between 100-250 bed volumes. Sol-gel incorporated with biomass only (+28/- 20), had maximum U-uptakes of 25-30 mg/g biomass and showed up and down swings with alternate cycles (Figure 16) to the end of the 8 cycles test. Sol-gel made with half fines had maximum U-uptakes of 21 mg/g and
29 mg/g biomass in cycles 4 and 6 (respectively) only, with a breakthrough point occuring in the first 50 bed volumes then dropping drastically to zero uptake in the third cycle and more gradually in the first cycle (Figure 17). Sol-gel formed with 50% fines and reduced ethanol showed the highest U-uptake values, consistently through 8 cycles between 15 mg/g to 40 mg/g biomass, with the breakthrough point at 50-100 bed volumes (Figure 18). All three sol-gel preparation were preconditioned, however, the first 250 bed volume load and 30 bed volume elution was low in U-uptake compared to the subsequent cycles.

Uranium elution from the U-loaded sol-gel particles followed a fairly predictable pattern through the above three tests. The first 10 bed volumes of 5% Na_2CO_3 passed through the column removed the highest amount of the U adsorbed, up to 5-10 times the amount in the second and third 10 bed volumes (Table 5). By the 30th bed volume U-elution was less than 10% of the first 10 bed volumes.

Uranium loading rates fluctuated considerably between 10 mL/min (0.6L/hr), 6.66 mL/min (0.4L/hr), and 3.33 mL/min (Figure 14, 15) U-uptake values of the sol-gel particles for one cycle, before plugging occurs, are 0-2 mg/g biomass, while at 3.33 mL/min (Figure 13), before plugging occurs, U-uptake values are as much as 26 mg/g biomass. At 6.66 mL/min (Figures 16-18), Uranium loads at between 5-20 mg/g biomass for the first cycle then shows relative increases in the subsequent cycles.

Increases in sol-gel mass were observed for 3 different stir tank tests; decreases in sol-gel mass for 2 stir tests and 1 column test; and no change in sol-gel mass for 2 other column tests (Table 4). After rotapping the recovered sol-gel, percentages of the recovered size to the final sol-gel mass were determined. There were increases in the +20/-14 fraction (0.02 g) of a +20/-14 size test, as well as the presence of 19% and 12% of the total final mass of a +28/-20 stir test being +20/-14. Eight cycle column tests using +28/-20 particles developed 4% and 18% of the final mass as +20/-14 while the other 8 cycle column test using +20/-14 particles had 38% of the fully recovered initial mass degraded to 22% (+28/-20) and the remainder fines. The tests done with reduced ethanol-formed +28/-20 particles resulted in less recovery of the +28/-20 size than those done with ethanol or the absence of ethanol in the formation. Testwork with +20/-14 had greated reduction of particles size in the column than in the stir tank.

4. DISCUSSION

Growth of the Rhizopus arrhizus biomass in 4.5 L quantities does not present the problems experienced growing the fungus in 200 mL quantities. The aeration and stir speed are more efficiently controlled, capable of supplying the increased oxygen requirements of this highly aerobic organism when grown for this particular purpose. The shorter growth period (24-30 hours compared to 30-40 hours) offers further streamlining of this stage of the process.

Disrupting the cell wall to release the cytoplasmic contents of this fungal species was not totally successful using osmotic shock methods. Experimental results of assaying for protein in solution following osmotic treatment indicate at the most, only half of the expected protein concentration in solution. The tests were conducted with 2.5 g of wet weight cells or about 0.5 g dry weight, which would produce a theoretical 125 mg to 200 mg protein based on the average protein composition of molds being 25-40% (dry wt. basis) (Weinshank, Garver, 1967). With protein values ranging from 17 mg to 80 mg after suspending the osmotically sensitive cells in distilled water, it is clear that isolating cell walls from Rhizopus arrhizus is not a simple procedure. A variety of techniques have been described in the available literature for breaking the tough and resilient fungal mucelium. These include ultrasonics, grinding, (hand, ball mill, bead mill). French pressure cell, Hughes press, chemical autolysis and enzymic lysis (Wiseman, 1969; Zetelaki, 1969).

The use of freeze dried biomass in sol-gel production is seen as having a distinct advantage over oven dried biomass. The consistency of the sol-gel product is a more homogeneous blend of biomass and sol-gel, making for a potentially easier particle sizing and less degradation or plugging during loading-elution in a column environment. The uranium uptake was higher per gram biomass incorporated into the sol-gel indicating perhaps some destruction of the molecular structures responsible for uranium adsorption during the oven drying process.

Adding comminution fines to the sol-gel formula does have a positive effect on the U-uptake. For sol-gel prepared with fines, the +28/-20 particles had U-uptake approaching 60 mg/g biomass through cycles 2 and 3 of a 3 cycle test (Figures 3-8), with an increasing trend shown in the 50% fines preparation. This is in contrast to sol-gel particles formed without fines (Figures 10-12), where U-uptake showed less than 30 mg/g biomass.

The low U-uptake values associated with cycle 1 suggest that the preconditioning step is not fully optimized. Particulate matter and particle size reduction are evident during the initial steps of preconditioning indicating damage to the sol-gel in both stir and column testwork may be expected. Reducing or removing the ethanol from the sol-gel formula lowers the degradation of the respective particle sizes as shown in Table 4. It also increases the U-uptake capacity of the sol-gel biomass (Figures 12 and 18), although it is noted that stir tests are compared to column tests. The potential does exist, however, for further refinement of the process in order to obtain higher U-uptake values.

Carbonate (5% w/v) elution of the U-loaded particles was optimal using sol-gel formulated with ethanol and fines, as shown in the 5-cycle stir tank tests (Table 5). Eight cycle column tests using fifty percent fines in combination with reduced ethanol or no ethanol in the formulation presented elution results comparable to the above, indicating that a more complete mass balance is achievable with select technical changes.

Similar trends are present between sol-gel U-uptake and loading effluent pH. Increases and decreases in both are observed in many of the loading schemes. Eight cycle column testwork (Figures 16-18) shows radical pH swings in the first 5 to 6 cycles with a more stable pH developing through the final cycles. It is in the later cycles that U-uptake is characterized by more distinct trends, dependant on the sol-gel formulation.

Column plugging was a problem early in the investigation. One solution to the problem may have been a change in the sol-gel formulation. The exclusion of ethanol or alternatively vacuum drying the sol-gel were two changes incorporated.

Fine white particulate matter, observed after contact with the carbonate elutant, was likely aluminum (See Results) and not likely silica. The change in the sol-gel formulation, as described, either prevented this loss of aluminum or at least minimized it, to allow flow through the column for a minimum 8 cycles.

Breakthrough points were established for this testwork at between 50 and 100 bed volumes for U-loading and at 10-20 bed volumes for U-elution, in each column tested load-elution cycle (Figures 16-18). These values were established using flow rates of 6.66 mL/min (0.4L/hr) for loading and an elution flow rate of 3.33 mL/min (0.2L/hr). If carbonate elution does have a deleterious effect on the sol-gel particle size, a lower flow rate may be instituted, although extended contact times may be equally deleterious. By increasing the flow rate of the loading solution (ie. minewater) to 10 mL/min (0.6L/hr) (Figures 14-15), lower U-uptake was evident, indicating the need for a longer contact time between the sol-gel and the minewater.

The greatest amount of particle size stability, in combination with U-uptake values, is +28/-20. The column testwork with this size shows the least destruction after 8 cycles, especially when ethanol is absent (or reduced) and 50% fines are used in the sol-gel formulation. The smaller particle size would allow for more rapid penetration of the minewater or elutant, the variables to be improved upon including higher porosity and increase stability. With developments such as these a larger particle size may be more desirable as comparable results (U-uptake, stability) were observed in +20/-14 particles.

5. CONCLUSION

The testwork undertaken proves that is is possible to physically stabilize industrial food waste grown Rhizopus arrhizus in a sol-gel support medium and obtain U-uptake values.

Physical stability is optimized without ethanol (or reduced amounts) in the sol-gel formulation and can result in U-uptake values up to 60 mg/g biomass. Breakthrough points of 50-100 bed volumes (at 0.4L/hr) and 10-20 bed volumes (at 0.2L/hr) were established for loading and elution, respectively. A lifetime of 8 cycles (or more) may be expected of the sol-gel biomass without extentive plugging or degradation by incorporating the following measures:

1. Restricting or eliminating the amount of ethanol used in sol-gel biomass formation.

2. Utilizing the comminution fines in the sol-gel formulation. Up to 50% fines (of the solids) produced the best results.

Losses of sol-gel appear to be unavoidable, during preconditioning, however, ethanol reduction in the formulation allows for reduced loss.

The loading elution capacity of the sol-gel biomass is less than the original pure biomass, with maximum loading capacity being 60%-70%. The sol-gel formulation may require optimization in this regard. Improvement to this process may yet be considered to maximize the removal of U from the bioleached minewater.

These includes scale-up procedures using large quantities of immobilized biomass. Full fluidization potential could be realized as could flow rate and flow direction.

The development of a pilot scale operation would allow for a comparison with existing ion exchange technology and identify weaknesses or strengths.

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TABLES

Table 1

Protein Concentrations Resulting
From Osmotic Shock of Rhizopus arrhizus

Treatment (see Fig. 2)	OD 280 nm	OD 260 nm	F	[Protein] mg/mL of sample	[Protein] total mg with dilution factor
S1	2.4	5.2	--	--	--
S2	0.410	0.510	0.944	0.388	77
S3	0.270	0.440	1.081	0.292	29
S4	0.611	0.670	0.852	0.521	80
S5	0.700	0.800	0.899	0.629	31
S6	0.480	0.490	0.814	0.391	78
S7	0.443	0.490	0.852	0.378	19
S8	0.350	0.485	1.023	0.358	54
S9	0.370	0.420	0.899	0.333	17
S10	0.450	0.505	0.870	0.395	59

OD= Optical density

F= Function of ratio OD_{280}/OD_{260} (as given by Layne, 1957)

[Protein] (mg/mL) = $F \times {}^{1}/_{d} \times OD_{280}$ nm (d=cuvette width in cm)

dilution factor = see Figure 2

TABLE 2

Sol-Gel Biomass Compositions and Comminutions

Comminution Method	Pure Biomass (g)	Fines (g)	Total Biomass (g)	Ethanol in Formation (mL)	Dried Sol-Gel (g)	Percent Biomass (%)	Recovered Particle Size +20/-14		+28/-20	
							g	%	g	%
Shatter Box	10	- -	10	15	15.15	66	- -	- -	- -	- -
Shatter Box	20	- -	20	30	23.23	87	8.5	37	4.7	20
Shatter Box	10 (Oven dried)	- -	10	15	10.45	95	- -	- -	- -	- -
Mortar/Pestle	20	- -	20	30	23.17	86	9.6	41	4.4	19
Mortar/Pestle	10	- -	10	- -	12.63	79	6.04	48	2.61	20
Shatter Box	5	1	5.67	7.5	6.54	87	2.81	43	1.47	22
Shatter Box	5	3	7	10	7.73	90	3.29	43	1.73	22
Mortar/Pestle	5	5	9	15	12	75	6.35	52	2.14	18
Mortar/Pestle	5	5	9	5	1.85	83	5.17	48	2.29	21
Mortar/Pestle	5	5	9	- -	11.16	80	5.20	47	2.31	20

TABLE 3

Preconditioning

Initial Particle Size (US mesh)	Initial Amount g	Size After Preconditioning						Ethanol (mL) in Formation
		(+20/-14)		(+28/-20)		(-28)		
		g	%	g	%	g	%	
+20/-14	7.35	0.46	6	0.93	13	1.01	>14	15 m/10 g biomass
+28/-20	3.88	0	0	0	0	3.88	100	15 mL/10 g biomass
+28/-20	2.39	0	0	0	0	2.39	100	15 mL/10 g biomass
+20/-14	5.17	3.41	66	0.62	12	0.11	>2	5 mL/10 g solids
+28/-20	2.28	0.17	7	0.12	5	0.38	>17	5 mL/10 g solids
+20/-14	6.04	4.08	68	0.68	11	0.07	>1	None
+28/-20	2.61	0.12	5	1.47	56	0.39	>15	None
+20/-14	5.20	2.60	50	0.75	14	0.29	>6	None
+28/-20	2.31	0.27	12	0.81	35	0.42	>8	None

Table 4

Size Distribution

Type of Test	# of Cycles	Initial Mass (g)	Final mass (g)	% increase decrease + / -	Initial Size (U.S. mesh)	Final Size (U.S. mesh)				Ethanol in Formation
						+20/-14		+28/-20		
						(g)	% of final mass	g	%	
Stir	10	0.50	0.43	-14	+28/-20	- -	- -	0.34	79	√
Stir	8	0.50	0.58	'+16	+20/-14	0.52	90	0.06	10	√
Stir	10	0.20	0.26	'+30	+28/-20	0.05	19	0.21	81	√
Stir	3	0.20	0.18	-10	+28/-20	0.028	16	0.14	78	√
Stir	5	0.20	0.25	'+25	+28/-20	0.03	12	0.20	80	- -
Column	8	0.50	0.50	0	+28/-20	0.04	8	0.39	78	- -
Column	8	0.50	0.50	0	+20/-14	0.31	62	0.11	22	- -
Column	8	0.50	0.49	- 2	+28/-14	0.02	4	0.33	67	reduced to 5 mL/10 g solids

Table 5
Elution Characteristics

TEST	BED VOLS. NA2CO3 ELUTED	LOAD ELUTION CYCLES TOTAL URANIUM (mg)											
		loaded	eluted	loaded	eluted	loaded	eluted	loaded	eluted	loaded	eluted	loaded	eluted
+28/-20 Column 8 cycle Half Fines, Reduced Ethanol	0	7		12.50		9.60		13.55		11.25		6.25	
	10		5.40				3.70		3.80				
	20		1.80				2.70		1.30				
	30		0.63		8.10		0.84		0.52		6.30		6.90
	TOTAL	7	7.83	12.50	8.10	9.60	7.44	13.55	5.62	11.25	6.30	6.25	6.90
+20/-14 Column 8 cycle Half Fines No Ethanol	0	2.75		5.35		3.75		11.75		3.75		6.25	
	10		3.10		4.10								
	20		0.78		0.92								
	30		0.37		0.48		3.60		3.90		3.00		1.41
	TOTAL	2.75	4.25	5.35	5.50	3.75	3.60	11.75	3.90	3.75	3.00	6.25	1.41
+28/-20 Column 8cycle No Fines No Ethanol	0	8.35		11.70		8.35		7.40		8.25		7.50	
	10		4.10				3.90		3.20		4.30		
	20		0.88		5.40		1.30		0.99		0.55		
	30		0.32		0.51		0.50		0.44		0.34		2.30
	TOTAL	8.35	5.30	11.70	5.91	8.35	5.70	7.40	4.63	8.25	5.19	7.50	2.30
STIR TESTS	TOTAL VOLUME (mL)	LOADED	ELUTED	LOADED	ELUTED	LOADED	ELUTED	LOADED	ELUTED	LOADED	ELUTED	LOADED	ELUTED
5 Cycles Half Fines +20/-14	0	1.0		8.6		11.9		8.7		9.8			
	30		0.57		2.1		2.43		0.48		0.42		
5 Cycle 3 g fines/5 g biomass +20/-14	0	0		10.50		8.70		8.40		8.70			
	30		0.78		11.70		9.00		7.80		7.80		
5 Cycle 1 g fines/5 g biomass +20/-14	0	1.0		11.70		5.50		8.90		7.50			
	30		0.63		13.20		2.07		9.90		7.20		
5 Cycle Pure Biomass	0	8		16		8.4		20.8		16.4			
	50		7.5		14		19.5		21		16.5		

FIGURES

Figure 1

Growth of Rhizopus arrhizus in the Braun Biostat V Fermentor as Monitored by pH

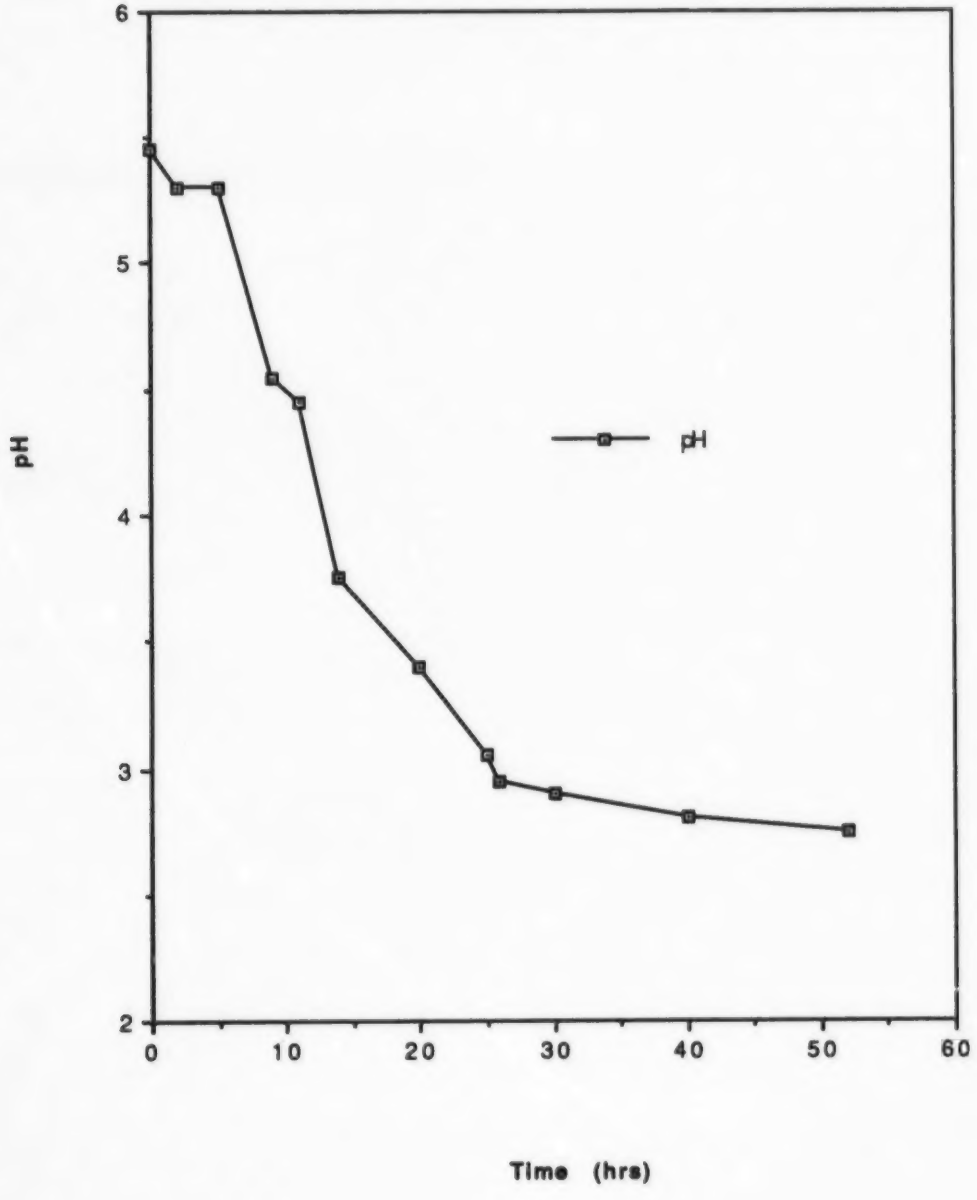


Figure 2

Protocol for Osmotic Shock of Rhizopus arrhizus Cells.

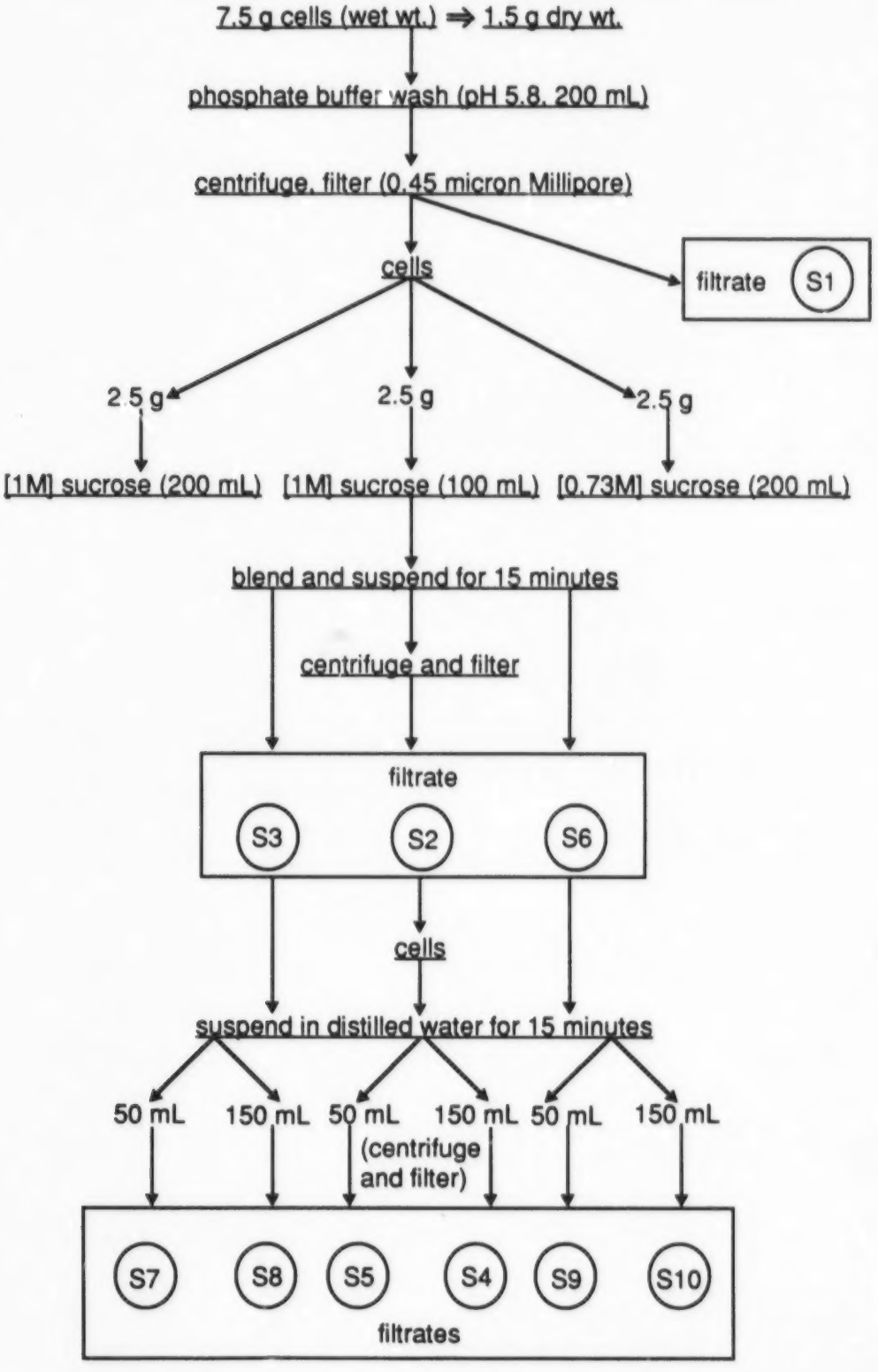
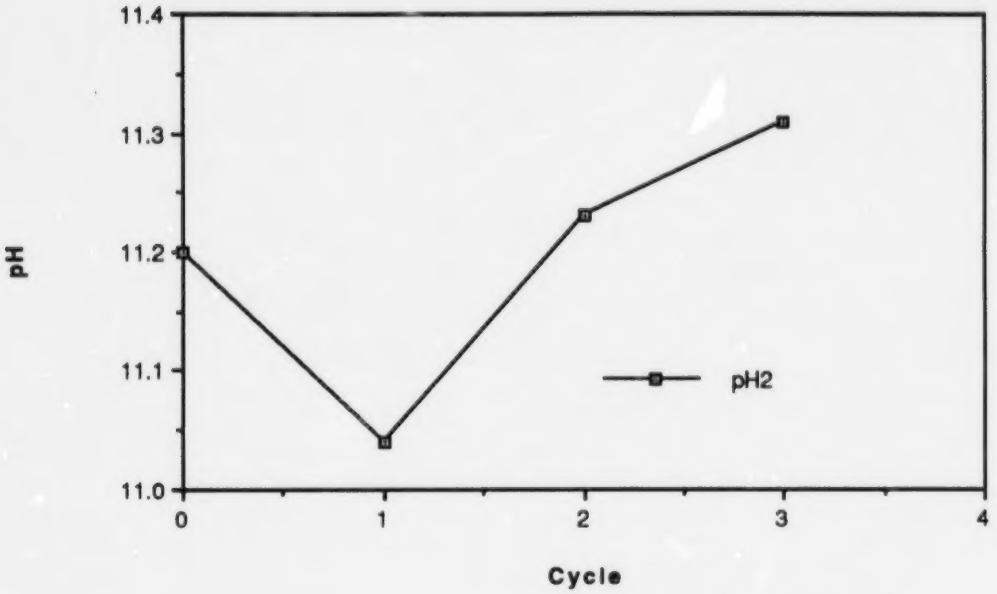


Figure 3
Effect of 3 Stir Tank Loading Elution Cycles on (+28/-20) Sol-Gel Particles (with 1 g fines, 5 g biomass) Elution pH



Effect of 3 Stir Tank Loading Elution Cycles on Uranium Uptake of (+28/-20) Sol-Gel Particles (with 1 g fines, 5 g biomass) and Loading Effluent pH

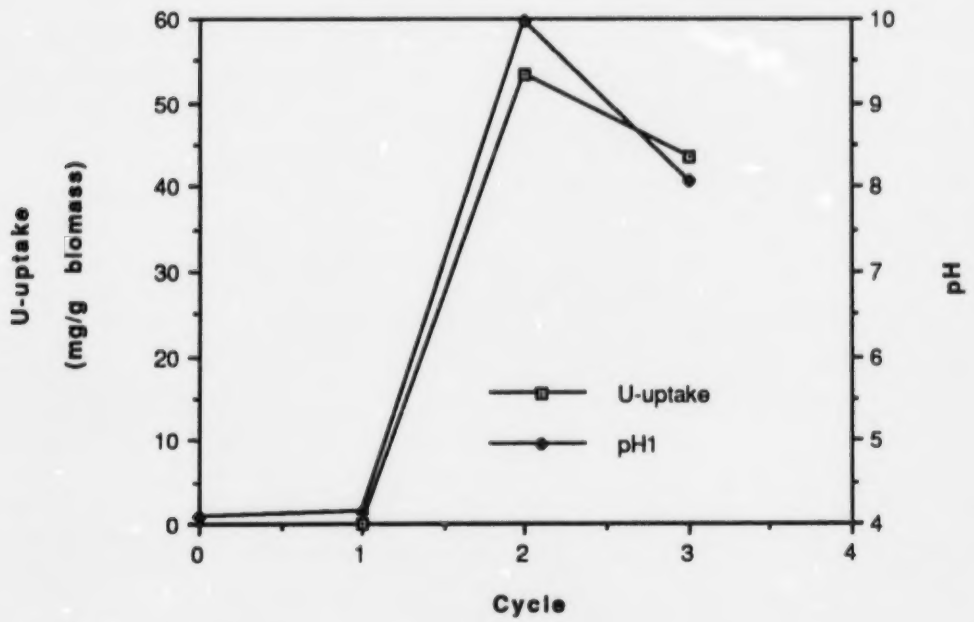
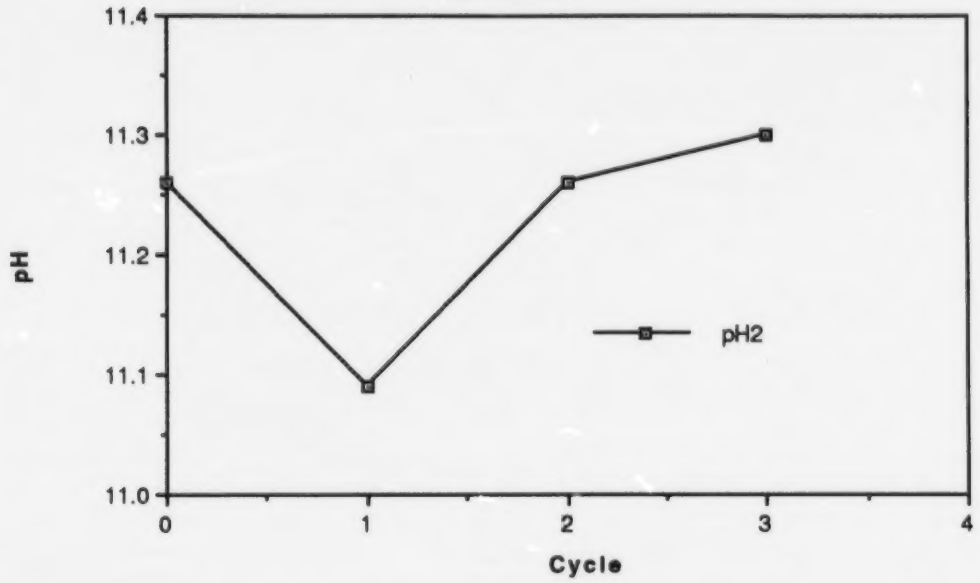


Figure 4
Effect of 3 Stir Tank Loading Elution Cycles
on (+28/-20) Sol-Gel Particles (with 3 g fines, 5 g biomass)
Elution pH



Effect of 3 Stir Tank Elution Cycles on Uranium Uptake
of (+28/-20) Sol-Gel Particles (with 3 g fines, 5 g biomass)
and Loading Effluent pH

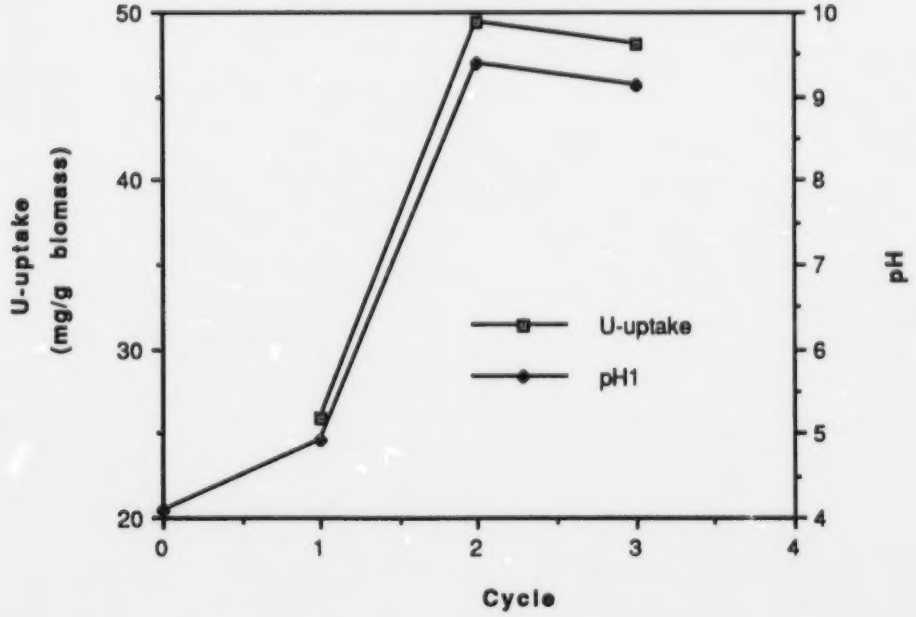
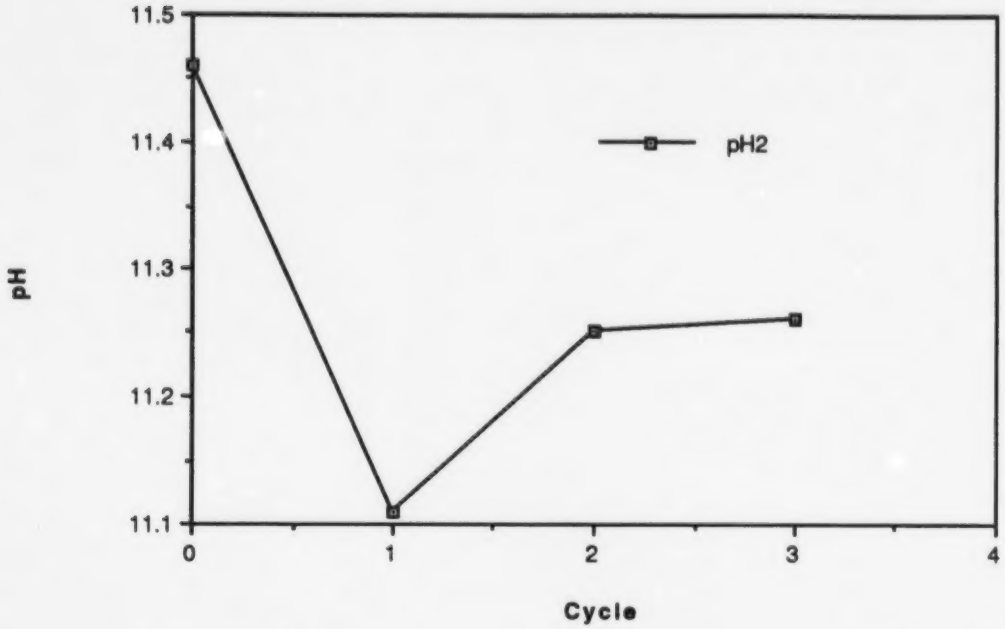


Figure 5

Effect of 3 Stir Tank Elution Cycles on (+28/-20) Sol-Gel Particles (with 5 g fines, 5 g biomass) Elution pH



Effect of 3 Stir Tank Loading Cycles on Uranium Uptake of (+28/-20) Sol-Gel Particles (with 5 g fines, 5 g biomass) and Loading Effluent pH

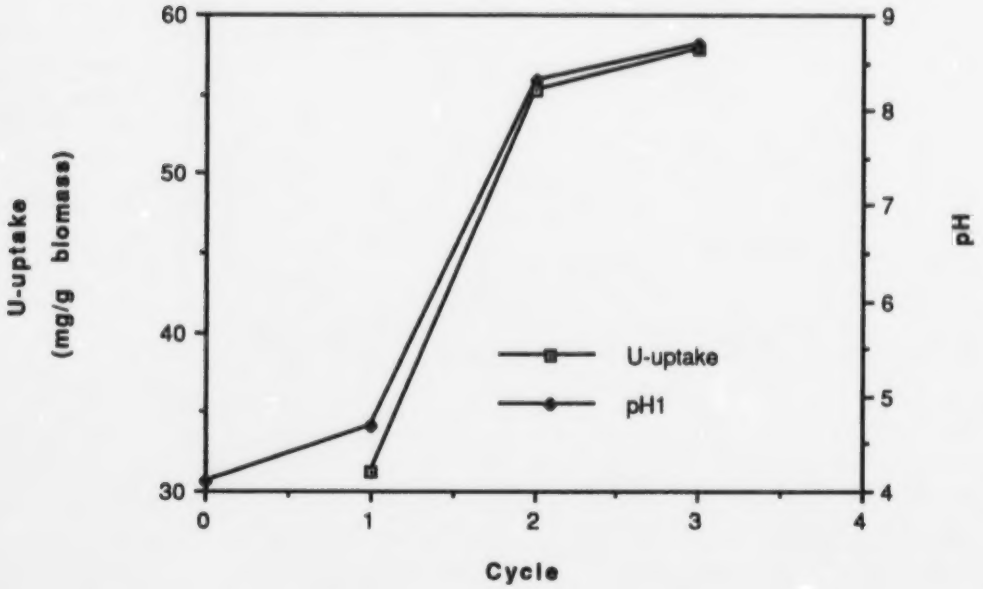


Figure 6

Effect of 5 Load/Elution Stir Tank Cycles on U-uptake of (+20/-14) Particles (with 1 g fines, 5 g biomass) and Loading Effluent pH

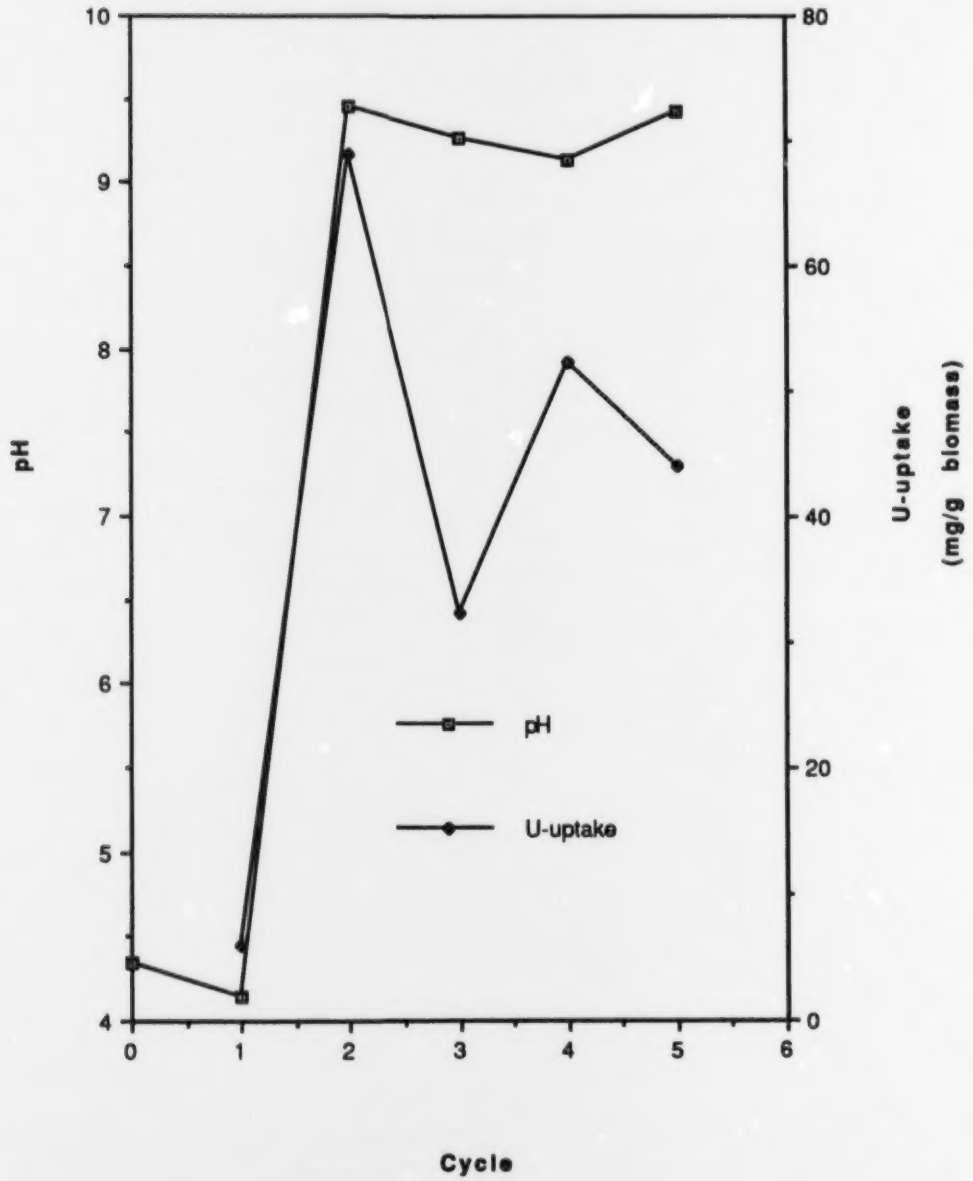


Figure 7

Effect of 5 Load/Elution Stir Tank Cycles on U-uptake of (+20/-14) Particles (with 3 g fines, 5 g biomass) and Loading Effluent pH

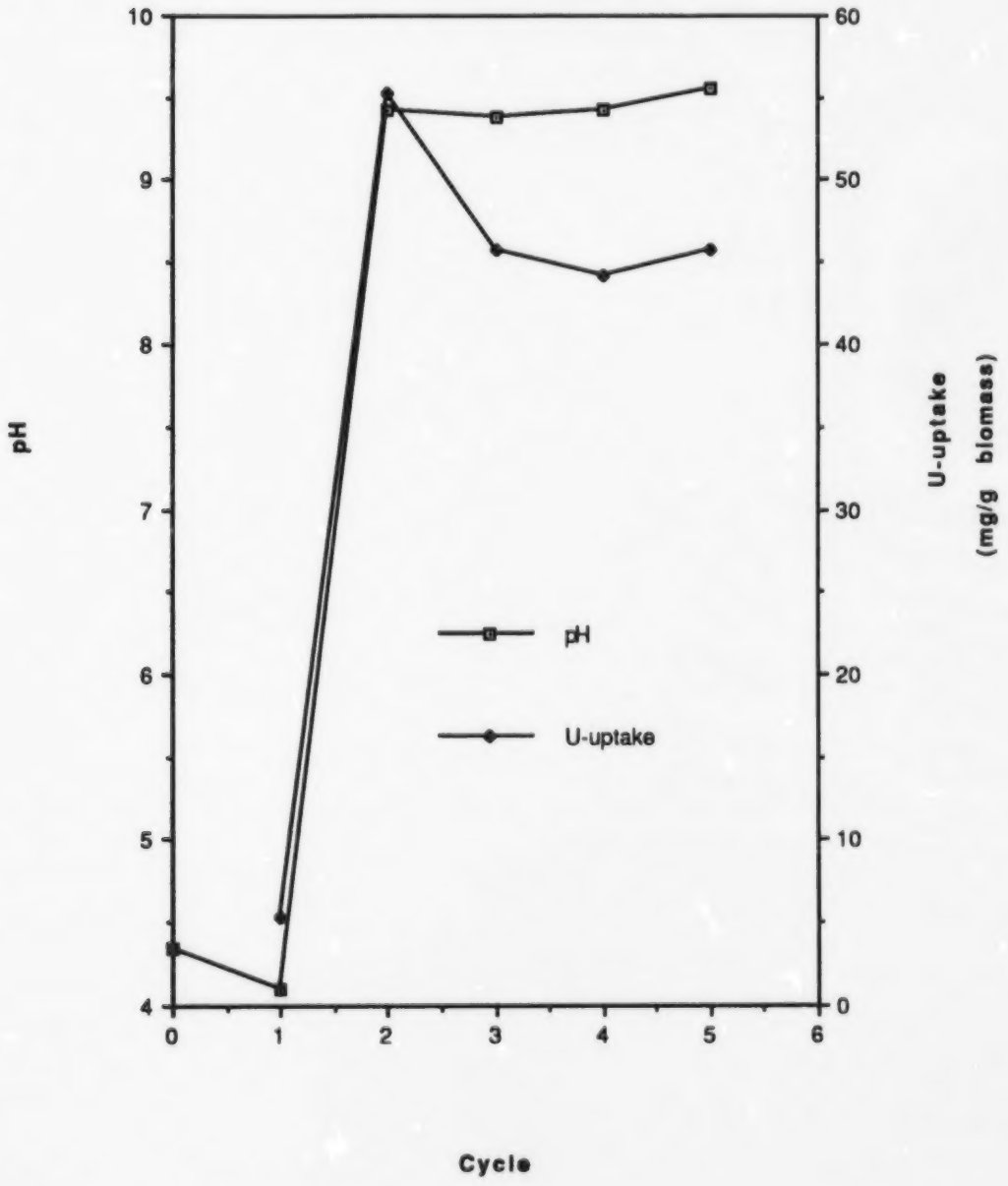


Figure 8

Effect of 5 Load/Elution Stir Tank Cycles on U-uptake of (+20/-14) Particles (with 5 g fines, 5 g biomass) and Loading Effluent pH

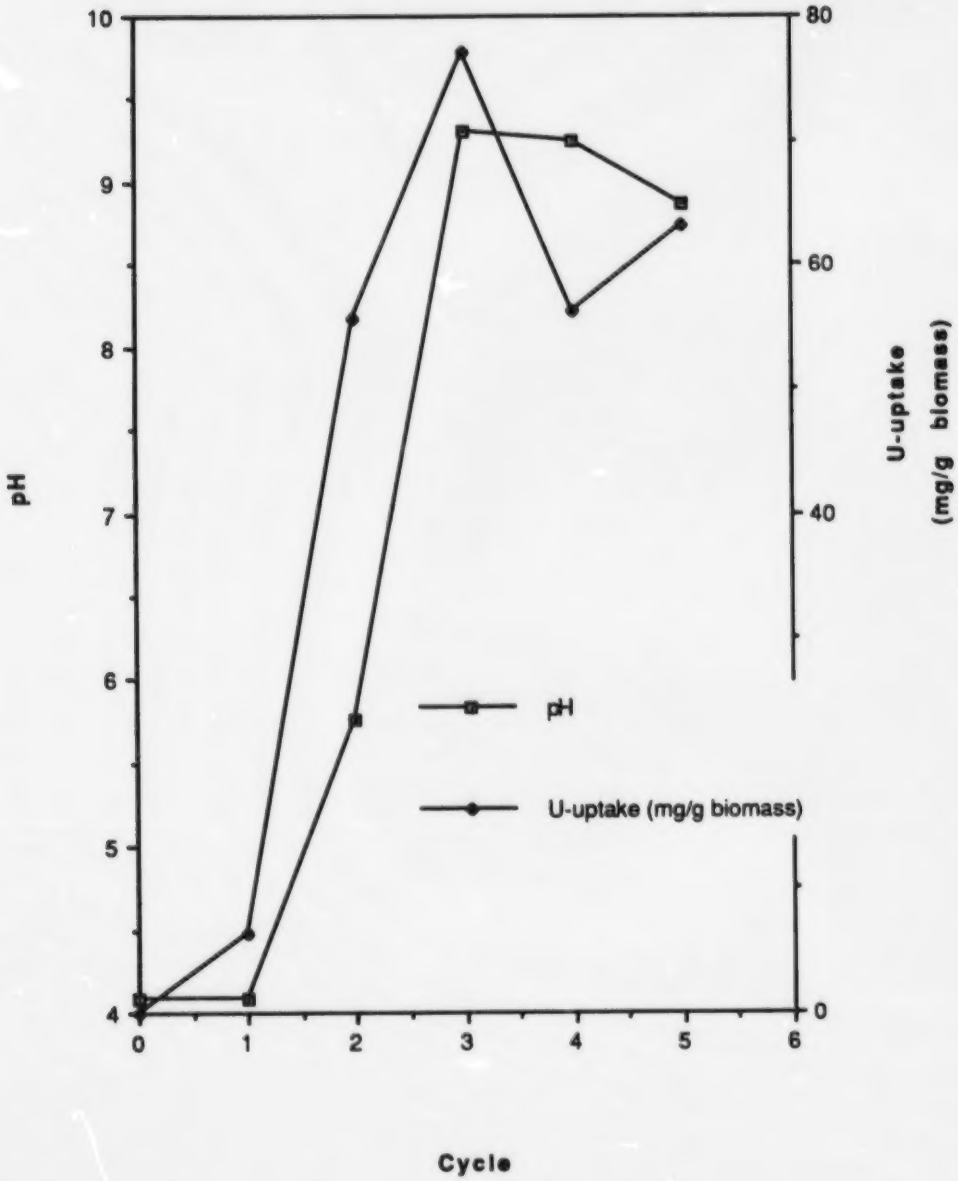


Figure 9

Effect of Load/Elution Stir Tank Cycles on U-uptake of Pure Biomass and Loading Effluent pH

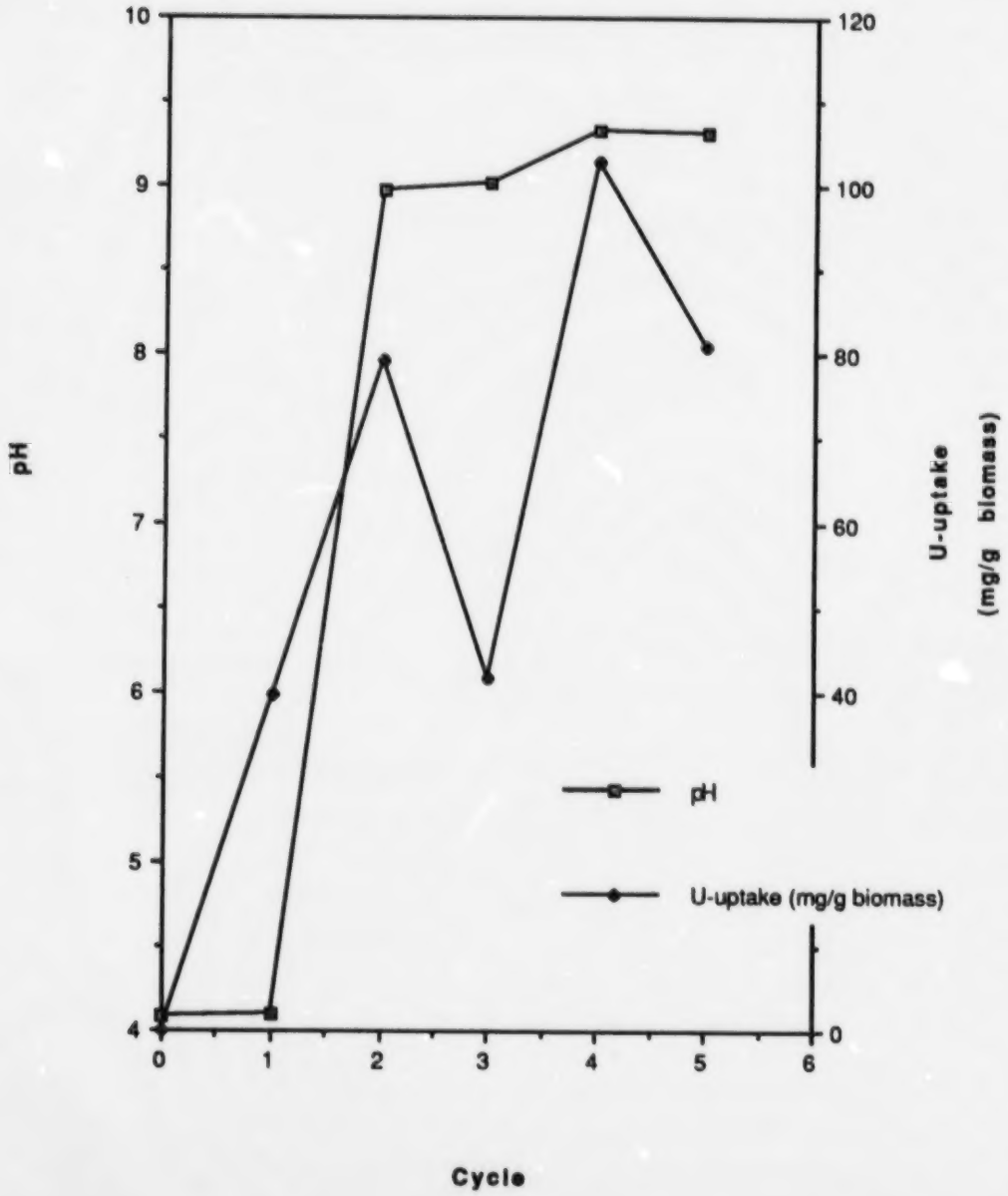


Figure 10

Effect of 8 Stir Tank Cycles on Uranium Uptake of

(+20/-14) Sol-Gel Biomass Particles and Solution pH

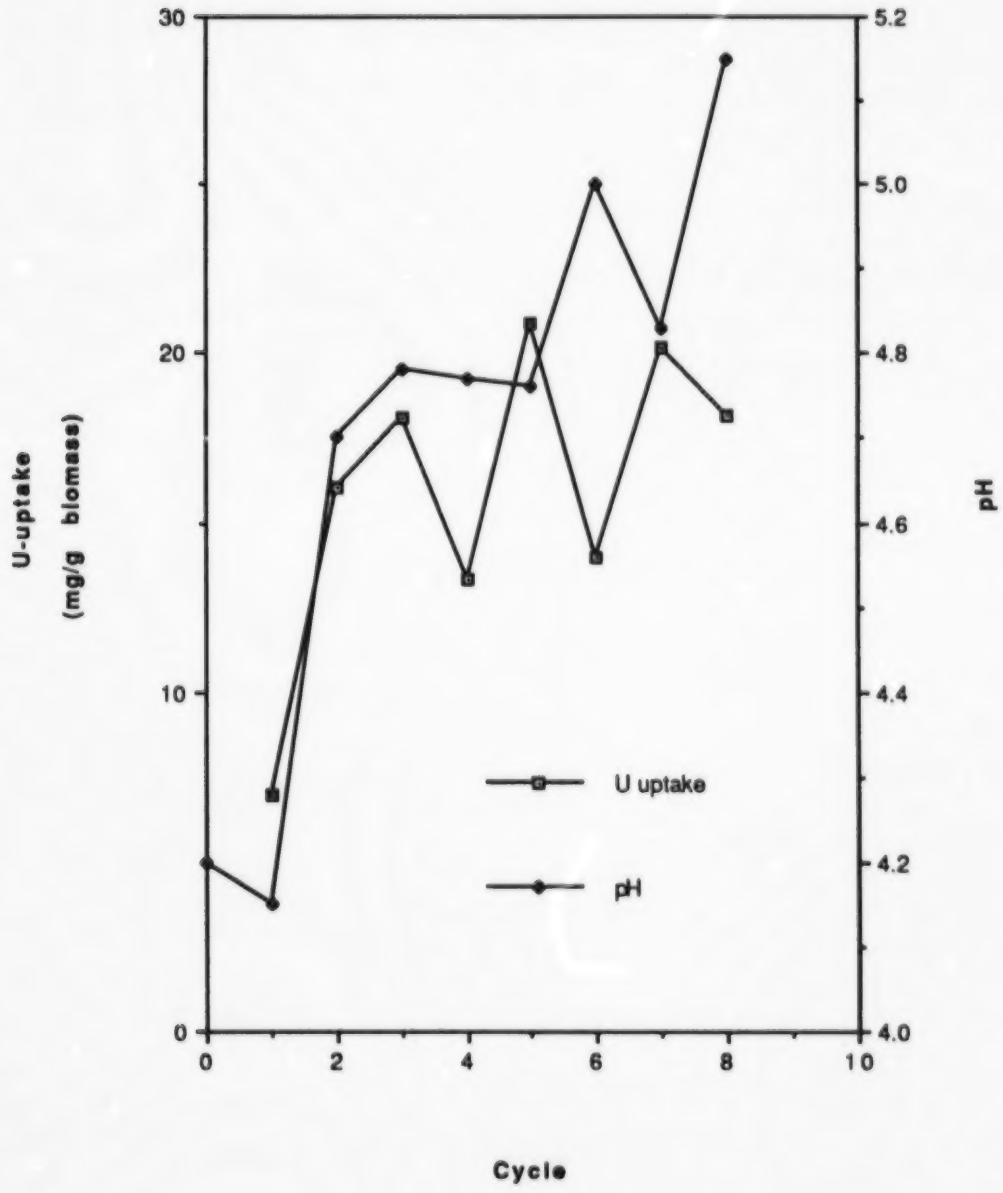


Figure 11

Effect of 10 Loading Elution Stir Tank Cycles on Uranium Uptake of (+28/-20) Sol-Gel Biomass Particles and Solution pH

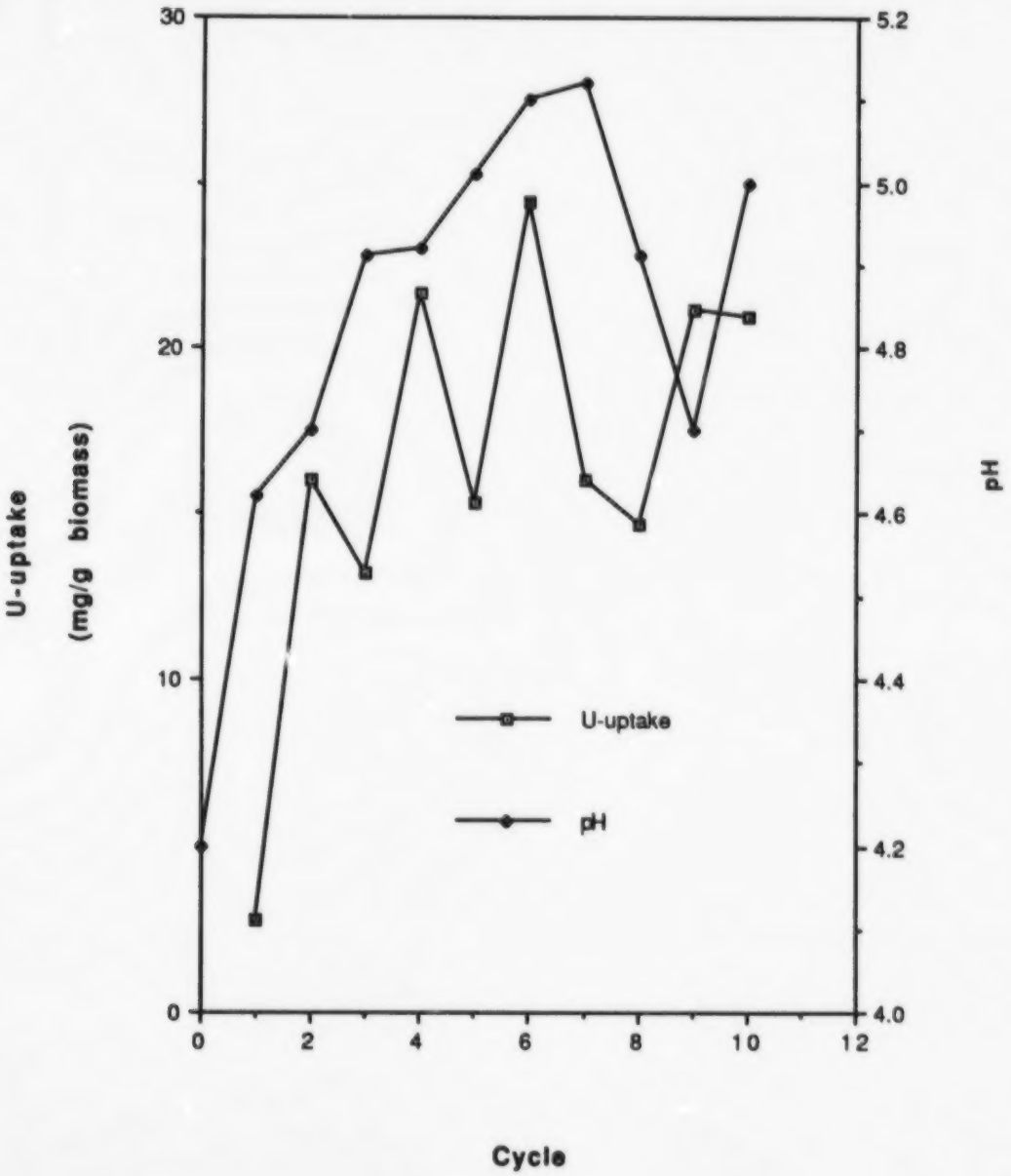


Figure 12

Effect of 10 Load/Elution Stir Tank Cycles on U-uptake By +28/-20 Preconditioned Sol-Gel Biomass (with Ethanol Formation) and the Effluent pH

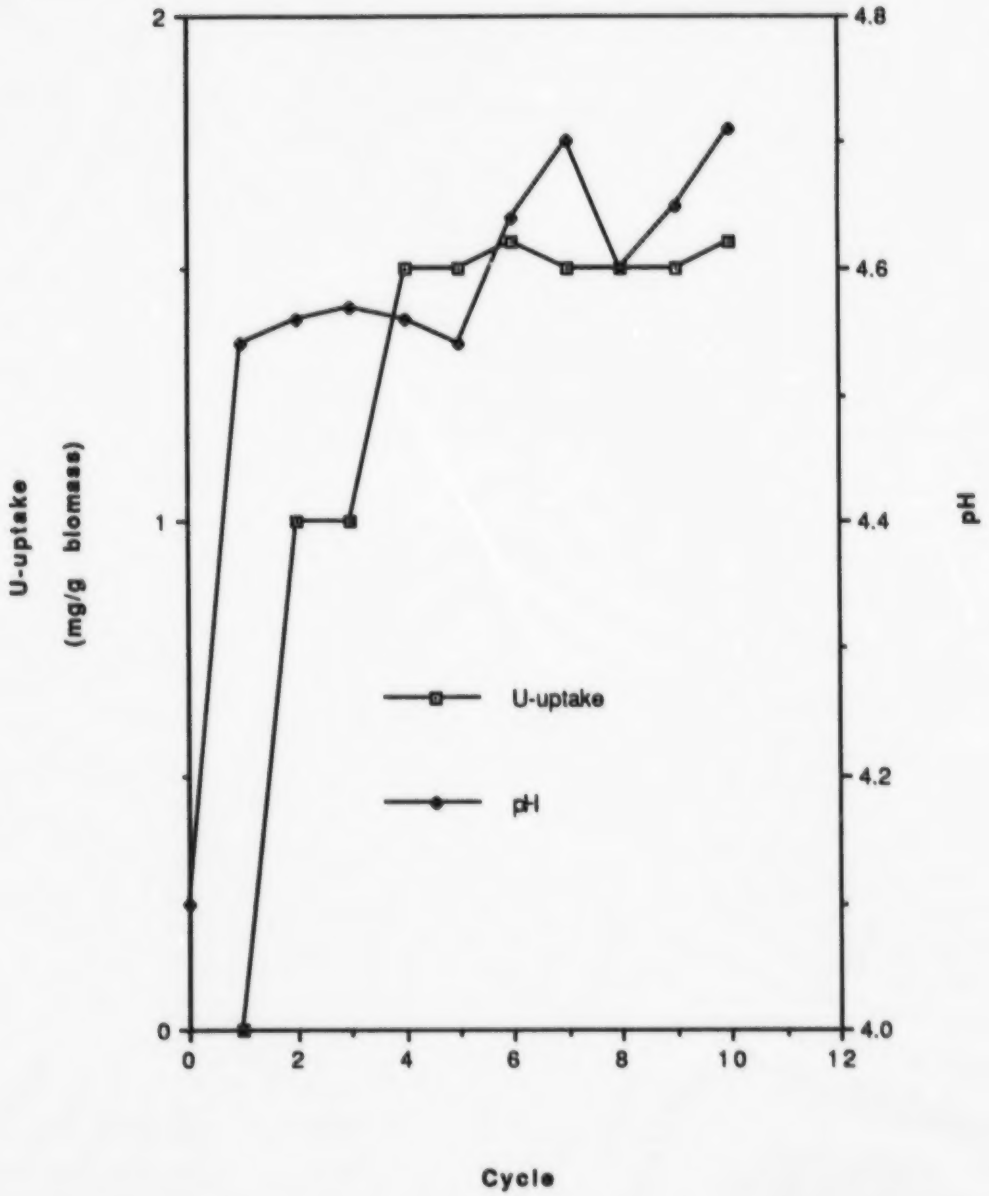
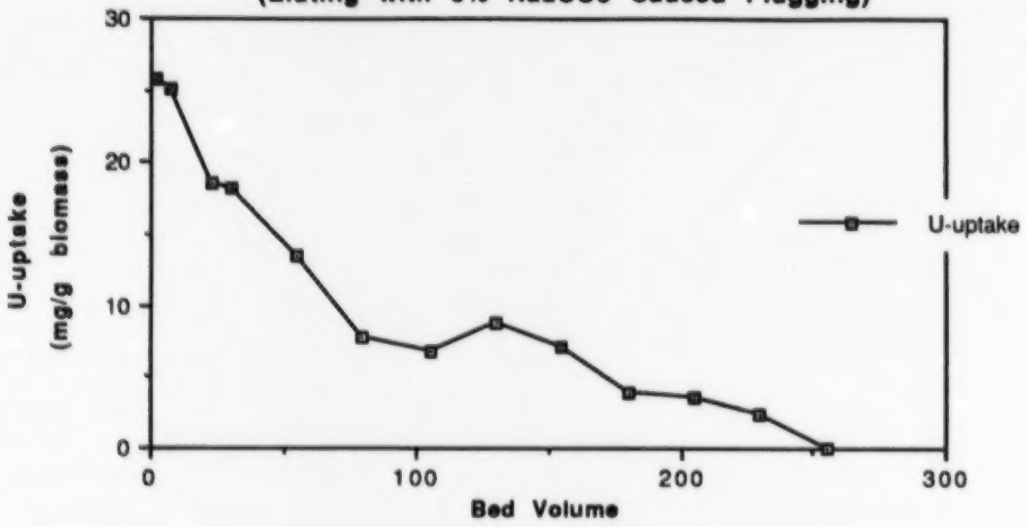
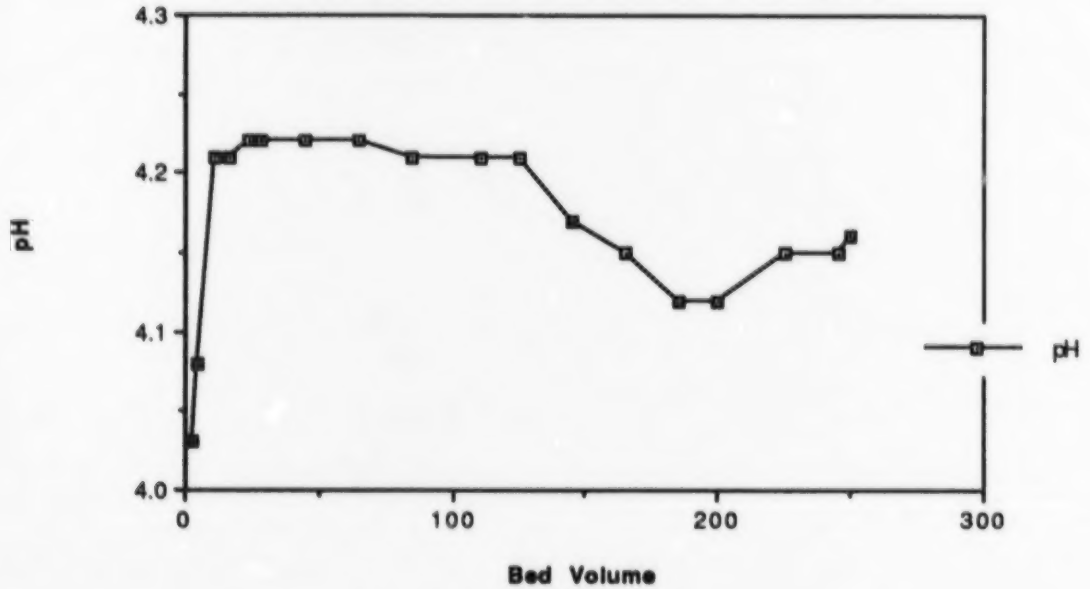


Figure 13

Column Loading Effluent pH Profile of (+28/-20) Sized Biomass Sol-Gel For One Cycle (2550 mL) (Eluting with 5% Na2CO3 Caused Plugging)



Column Loading Profile of (+28/-20) Sized Biomass Sol-Gel For One Cycle (2550 mL) (Eluting with 5% Na2CO3 Caused Plugging)

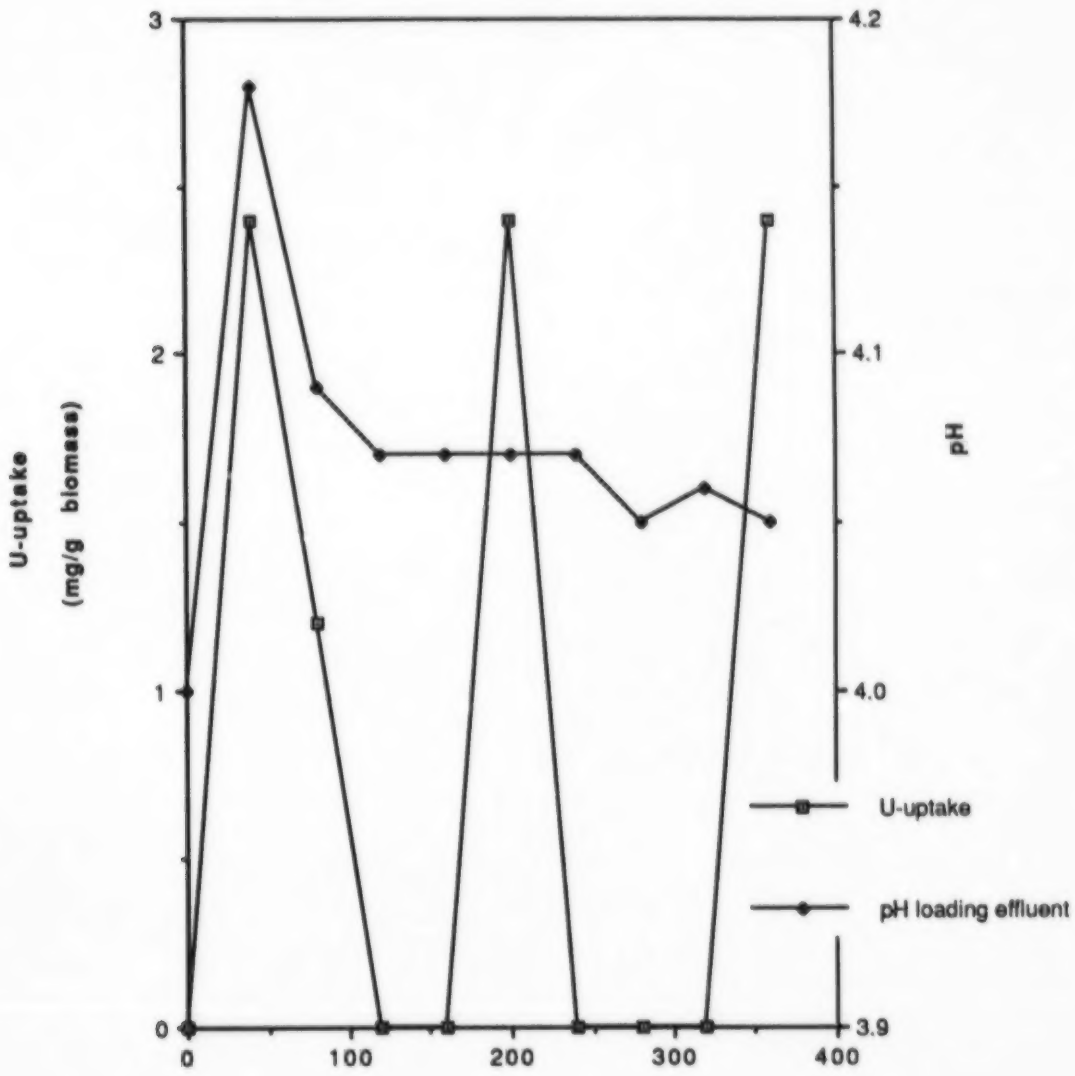


Bed Volume:	10 mL
Total Sol-Gel:	6.23 g
Load Flow Rate:	3.33 mL/min (0.2L/hr)
Column Width:	1 cm

Figure 14

Column Loading Profile (+20/-14) Sol-Gel

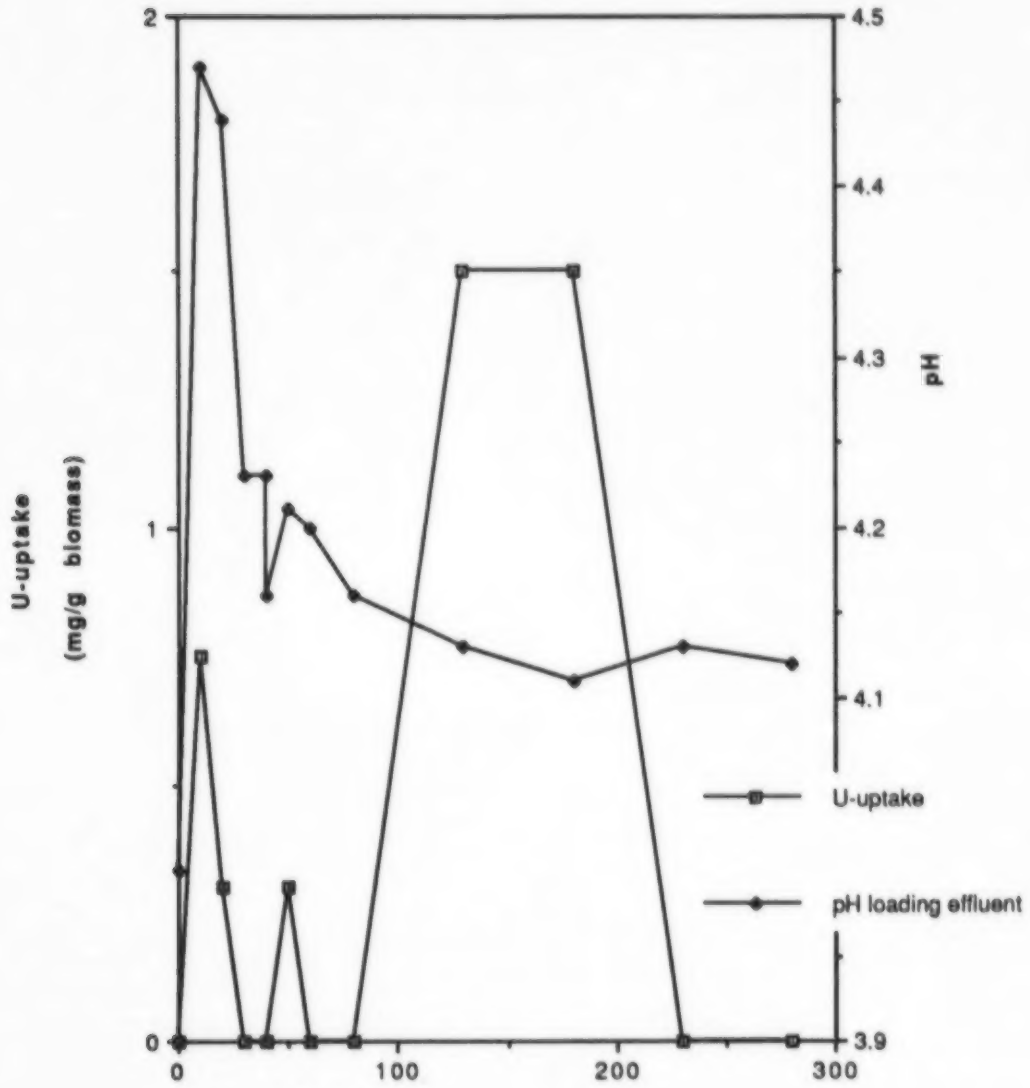
Particles For One Cycle Before Plugging Occurs



Bed Volume: 1 mL
Total Sol-Gel: 0.5 g
Load Flow Rate: 10 mL/min
Column Width: 0.5 cm

Figure 15

Column Loading Profile of (+20/-14) Sol-Gel Particles For One Cycle Before Plugging Occurs



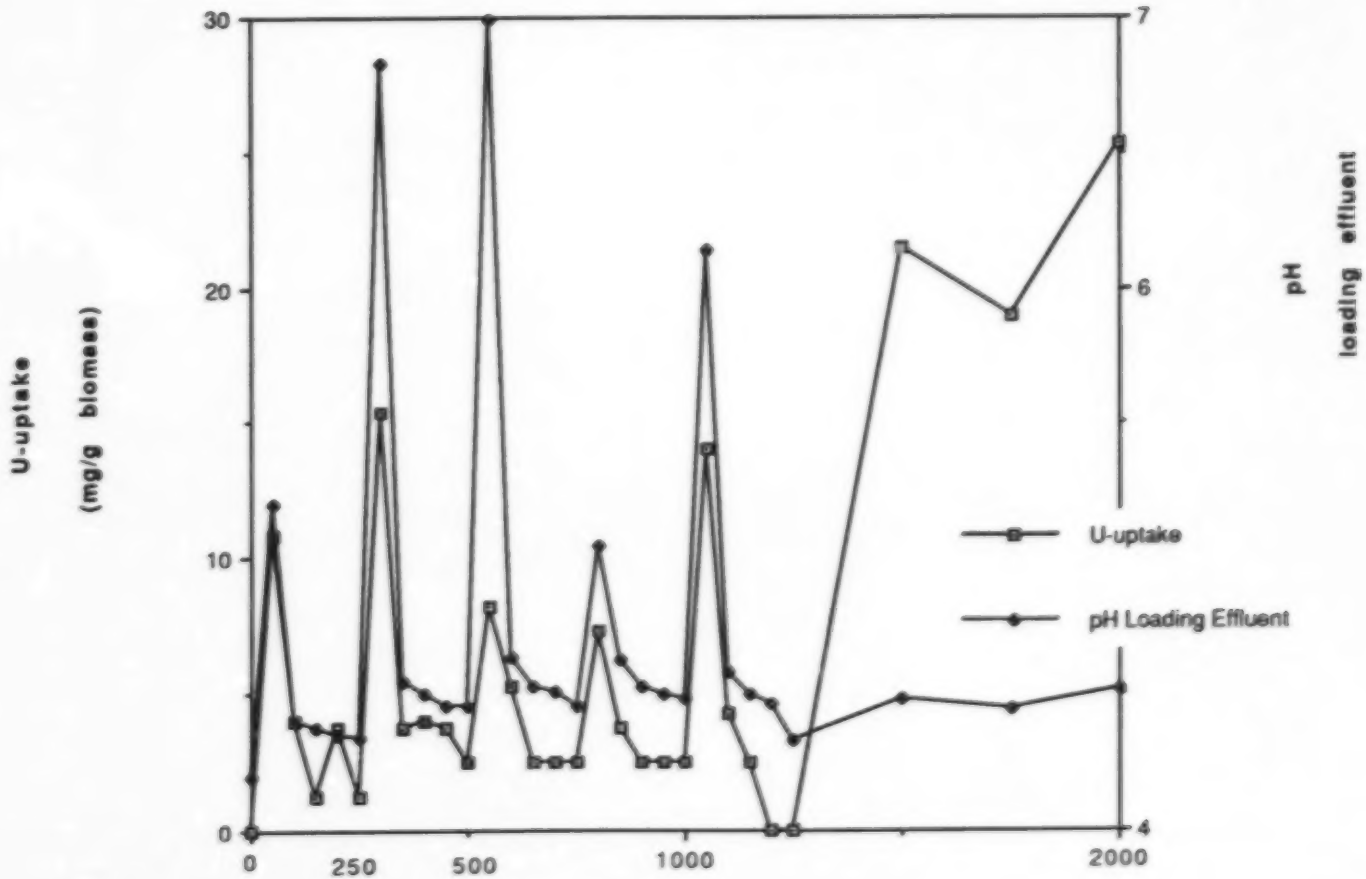
Bed Volume: 1 mL
Total Sol-Gel: 0.5 g
Load Flow Rate: 10 mL/min
Column Width: 0.5 cm

Figure 16

Effect of 8 Load/Elution Cycles in a

Column on U-uptake by +28/-20 Sol-Gel Particles

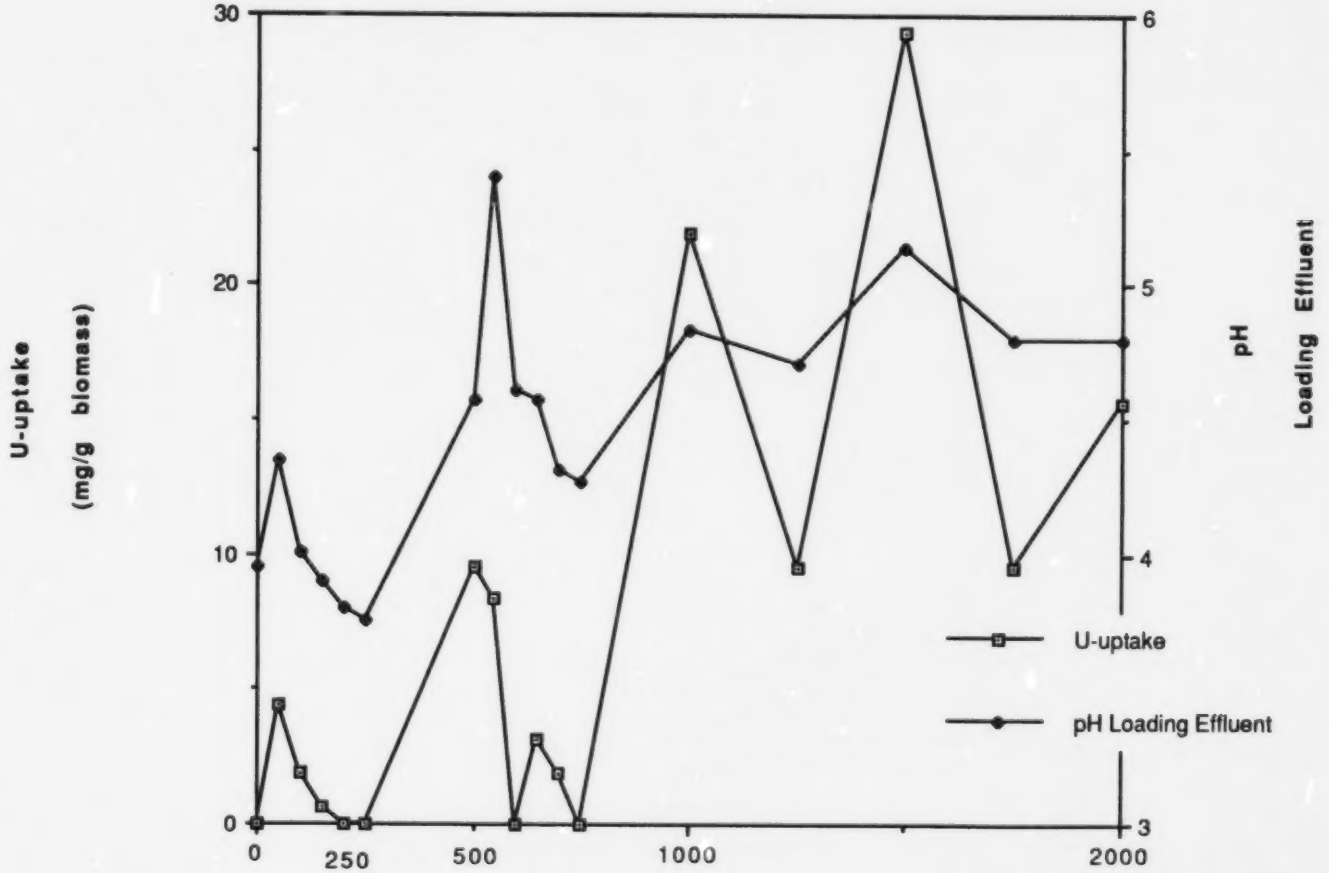
Without Ethanol or Fines in the Formation



Flow Rate: 0.4L/hr.

Figure 17

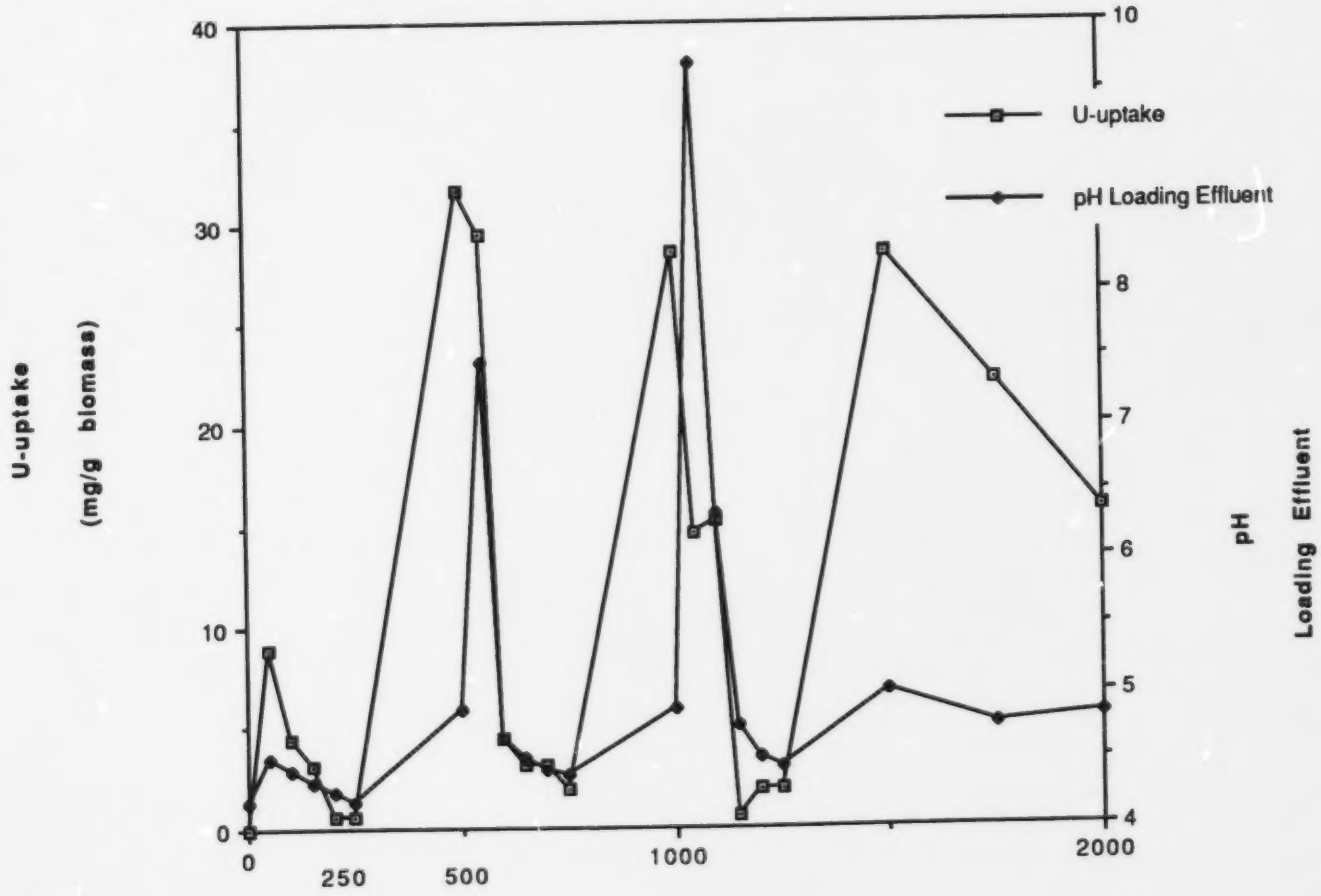
Effect of 8 Load/Elution Cycles in a Column on U-uptake by +20/-14 Sol-Gel Particles with 5 g fines/5 g biomass but no Ethanol in the Formation



Flow Rate: 0.4L/hr.

Figure 18

Effect of 8 Load/Elution Cycles in a Column on U-uptake by +28/-14 Sol-Gel Particles with 5 g fines/5 g biomass and 5 mL Ethanol/10 g solids in the Formation



Flow Rate: 0.4L/hr.

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CONTRACT PLAN AND REPORT FORM – FORMULE DE PROJET DE CONTRAT ET DE RAPPORT

DSS-MAS 9143-3 (4/81)

CONTRACT TITLE – TITRE DU CONTRAT

CONTRACTOR – ENTREPRENEUR

ORTECH 41-11255

DSS23440-9-9015/01-SQ

TASK DESCRIPTION / DESCRIPTION DE LA TÂCHE	TASK DURATION IN MONTHS – DURÉE DE LA TÂCHE EN MOIS	TASK COST – COÛT DE LA TÂCHE		
	Oct. '89 / Nov. '89 / Dec. '89 / Jan. '90 / Feb. '90 / Mar. '90 / 7 / 8 / 9 / 10 / 11 / 12	PLANNED / PRÉVU	ACTUAL / RÉEL	E.T.C. / À VENIR
Biomass Preparation (1.1)		8K	$10,000	Dec. 31/90
Sol-Gel Biomass Prep. (1.2)		on going	$ 8,000	Mar. 31/90
Stir Tank Tests (2.1)		5K	$ 5,000	Dec. 31/90
Biomass Comminution(2.2)		3K	$ 3,000	Mar. 31/90
Column Set-up (2.3)		8K	$ 5,000	Mar. 31/90
Column Elution Breakthrough (3.1)		7K	$ 5,000	Feb. 28/90
Flow Rate Optimization (3.2)		5K	$ 5,000	Mar. 31/90
Size Distribution (3.3)		5K	$ 2,356.74	Mar. 31/90

PLANNED COST BY MONTH / COÛT PRÉVU POUR CHAQUE MOIS	3K	5K	8K	10K	10K	5K	PLAN 41K	
ACT. / RÉEL – E.T.C. BY MONTH / PRÉVISION POUR CH. MOIS	4,727.91	3,519.47	2,663.20	4,085.11	6,469.25	19,889.80		ACTUAL COSTS TO DATE / COÛTS RÉELS À CE JOUR 41,356.74 / E.T.C. / À VENIR Mar. 31/90